Anabolic Steroid Induced Hypogonadism (ASIH)

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The recurring controversy and politicization on the use of anabolic androgenic steroids (AAS) has been front and center in the news headlines. Within the last month the release of the book, "Wada MF, Williams, L. Game of Shadows: Barry Bonds, BALCO, and the Steroids Scandal that Rocked Professional Sports. Gotham Books; March 23, 2006," precipitating Commissioner Bud Selig to name George J. Mitchell, former Senate majority leader, to lead an investigation into what appears to be the sport's long, and troubling, involvement with steroids. This falls almost exactly one year after publication of the book, "Canseco, J. Juiced: Wild Times, Rampant 'Roids, Smash Hits, and How Baseball Got Big. Regan Books; February 14, 2005." Following within a month was the Government Reform Committee Hearing, United States House of Representatives, on March 17, 2005. The hearing was entitled "Restoring Faith in America's Pastime: Evaluating Major League Baseball's Efforts to Eradicate Steroid Use."[[1]] The hearing was the first in a series of hearings regarding steroid use in professional sports.

Since the introduction of steroids into mainstream culture, the media, sports organizations, medical community and public have all expressed their values and judgment of their ethical use outside of medical necessity. Within the medical establishment there is a pervasive atmosphere of fear and intimidation towards physicians who treat AAS users or prescribe AAS. This has created a vacuum or void in the proper use of AAS; an abandonment of basic scientific principles; and an ever increasing population of men at risk for significant health problems. For the greater part of 10 years I have found that the medical treatment provided for the condition termed anabolic steroid induced hypogonadism (ASIH), is nonexistent or ignored by the great majority of medical professionals. As predicted since my entry into this field in 1995 more and more cases of ASIH would appear due to this negligence. Clear and convincing evidence of this is demonstrated by recent articles in peer-reviewed medical literature affirming concerns for the long term effects of untreated ASIH [[2]], rapidity and severity of symptoms in ASIH [[3]], and inappropriate treatment with AAS based upon a flawed clinical study design [[4]].

An unproven and unfounded assumption has been made in the medical establishment that the treatment for an individual suffering from ASIH is to do nothing which is coined 'watchful waiting' and in time HPTA functioning will return to normal. This premise can be traced back to Knuth et al. (1989) [[5]] studying semen parameters in AAS users. He concluded, "Results suggest that even after prolonged use of extremely high doses of anabolic steroids, sperm production may return to normal." The ability to create spermatozoa does not equate with a normal functioning HPTA. Hypogonadal males are known to have the ability to produce spermatozoa. There are no studies that demonstrate that serum testosterone levels sufficient for spermatozoa production are positively associated with the clinical effects of testosterone elsewhere within the individual. At the very same time members of the medical community announce an alert to suicide risk after AAS cessation. Kirk J. Brower, M.D. from the University of Michigan stated, "… whereas

depressive episodes and suicide attempts are most likely to occur within three months of stopping AAS use."[6] Shortly thereafter Texas HB 3563, "Use Of Anabolic Steroids By Public School Students," was passed and signed into law June 18, 2005. Of particular importance is the bill analysis citing the problem of "clinical depression when steroid use is stopped." [7] The obvious question is who are these astute physicians that are able to know the individual to attempt suicide during the treatment plan of 'watchful waiting' or do nothing?

AAS have proven beneficial in treating numerous medical conditions and symptoms in ailing populations. Currently HIV males account for an estimated 560,000 people in the U.S. reports the Centers for Disease Control and Prevention. Those experiencing lean muscle wasting greater than 10% will likely be administered a form of AAS therapy to help retain fat free tissue. According to the U.S. Food and Drug Administration, approximately 5 million men in the U.S. are considered hypogonadal with roughly 250,000 being treated with testosterone replacement. Finally, as of 2002 over 14 million men suffer with osteoporosis and low bone mass according to the National Osteoporosis Foundation. Cumulatively 810,000 people are possibly being treated with some type of androgen or AAS. Add to these patients the countless numbers of adolescents, young and middle aged men, and athletes using AAS for cosmetic and athletic enhancement the potential population of at risk men numbers well over one million.

ANDROGENIC ANABOLIC STEROIDS (AAS)

Testosterone and testosterone analogues, anabolic-androgenic steroids (AAS), have long been used in the athletic community for improving lean muscle tissue and strength. A positive correlation has been shown with testosterone to include: increased protein synthesis resulting in lean muscle tissue development [8], enhanced sexual desire (libido) [9], increased muscular strength [10], increased erythropoiesis [11], a possible positive effect on bone development [12], improved mental cognition and verbal fluency [13], and male masculinizing characteristics [14]. Recently, however, clinicians have recognized the potential benefits of their use in the treatment of various conditions and ailments. Numerous studies have discussed the use of AAS in the treatment of HIV-associated conditions [15], hypogonadism [16], impotence [17], burn victims [18], various anemia's [19], deteriorated myocardium [20], glucose uptake [21], continuous ambulatory peritoneal dialysis (CAPD) [22], alcoholic hepatitis [23], hemochromatosis [24] and prevention of osteoporosis [25]. Since there has been such strong evidence for the medicinal use of AAS in the treatment of various conditions, these medications have become more prevalent in the medical community.

While the use of AAS by physicians has become more prevalent, this class of medicines is not without their inherent problems. AAS have been shown to induce hypogonadotropic hypogonadism [26]. This condition typically results from an abnormality in the normal functioning of the hypothalamic-pituitary-gonadal axis (HPTA), either from an over-or underproduction of one of the hormone secreting glands, causing a cascading unbalance in the rest of the axis. This condition may be the result of a physiological abnormality (i.e. mumps orchitis, Klinefelters syndrome, pituitary tumor) or as an acquired result of

exogenous factors (i.e. androgen therapy, anabolic-androgenic steroid administration). Clerico et al found a dramatic suppression of serum gonadotropin levels in athletes given methandrostenolone, suggesting a direct action of AAS on the hypothalamus [[27]]. Similar results of suppressed gonadotropins have been found in patients supplementing solely testosterone [[28]]. Case report studies discussed a 36-year old male competitive bodybuilder and a 39-year old father, each using various AAS regimens over extended periods of time, who showed a blunted response to GnRH stimulation tests [[29]]. Bhasin et al showed a complete suppression of serum luteinizing hormone levels after administration of 600 mg testosterone enanthate over ten weeks [[30]]. A similar study administered 600 mg of nandrolone decanoate to 30 HIV-positive males over twelve weeks [[31]]. The results documented mild elevations in hemoglobin and alanine aminotransferase levels but no reference to LH or testosterone levels. The lack of gonadotropin response is puzzling as the data showed 12 of 30 subjects experienced testicular shrinkage, implying Leydig cell dysfunction and suppressed testosterone levels. A contraceptive investigation found that 6 of 9 men receiving 200mg of testosterone enanthate per week became azoospermic with suppressed gonadotropin levels after 16-20 weeks [[32]]. Other studies using AAS also showed no reference to LH or FSH levels but suppressed values are expected in each case [[33]].

Declining, or suppressed, circulating testosterone levels as a result of either pathophysiological or induced hypogonadal conditions can have many negative consequences in males. Declining levels of testosterone have been directly linked to a progressive decrease in muscle mass [[34]], loss of libido [[35]], decrease in muscular strength [[36]] impotence [[37]], oligospermia or azoospermia [[38]], increase in adiposity [[39]] and an increased risk of osteoporosis [[40]].

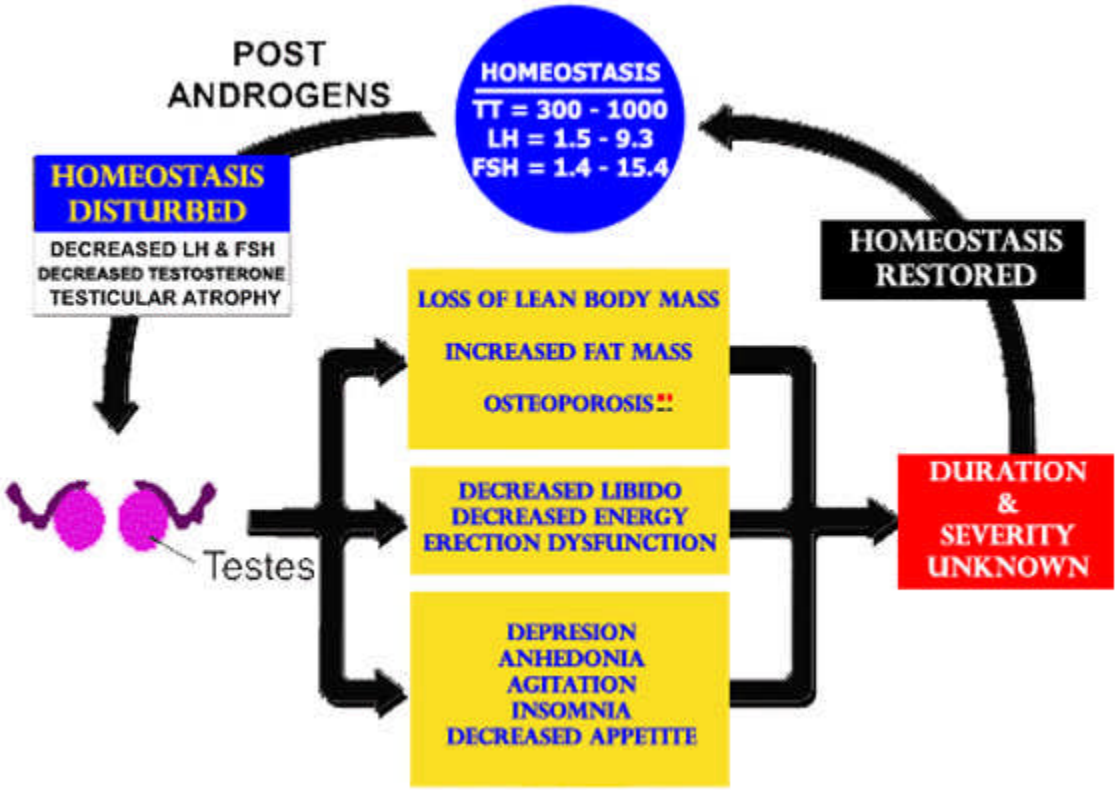
CHRONOLOGY

In 1982, more than two decades ago, it was shown that nandrolone decanoate caused a suppression of the HPTA in males. [[41]] A 1989 study demonstrated the period of hypogonadism after androgenic-anabolic steroid cessation in male hemodialysis patients.[[42]] The authors warned that the cessation of anabolic steroids caused hypogonadism stating: "Nandrolone decanoate are anabolic steroids prescribed for uremic anemia and those may possibly exacerbate uremic gonadal damage. This clinical study suggests that some anabolic steroids play a role in uremic hypogonadism."

The sequence of changes in body composition induced by testosterone and reversal of changes after cessation was studied in 1992. Testosterone treatment produced a progressive increase in lean body mass and a progressive decrease in body fat. After the testosterone was stopped a period of hypogonadism ensued and the body composition reverted slowly back to normal. [[43]]

Each of the studies done prior to 1995 is designed correctly taking into consideration the characteristics of life. The characteristics of life are to physiology as Newton's Laws of Motion and Gravity are to physics. If one was to disregard or fail to consider the Law of Gravity in a physics experiment the conclusions drawn from such a study would be

erroneous and wrong. The Characteristics of life are the following: All living things follow the tenets of cell theory; Living things acquire and use energy and produce wastes; Living things reproduce, grow, and develop; Living things evolve;. Living things respond to stimuli; Living things maintain a state of homeostasis; All living things are made up of some kind of atoms and molecules. The scientific method is a method of collecting evidence through observation, questioning, hypothesis formation, and hypothesis testing. Similarly, if one was to disregard or fail to consider the characteristics of life in a physiology experiment their conclusions would be erroneous and wrong.



THE FAILURE TO ACCOUNT FOR HOMEOSTASIS WOULD BE THE EQUIVALENT OF STATING GRAVITY DOES NOT EXIST.

MIND WARP

Ironically it was not until 1996 and the publication by Bhasin [[44]] that the medical community finally came to recognize that androgens do enhance the ability of the body to manufacture muscle. Bhasin failed to document and report the effects for the period following cessation of testosterone. Since its publication, the same experimental protocol with no follow up has been used in many patient populations by many different researchers. Uniformly publications have reported positive body composition changes with AAS administration but neglect to include any follow-up on duration and severity of ASIH after AAS cessation. [[45]]

That it took over 60 years since the discovery of the hormone testosterone and countless years of unsupported comments by the pundits of the exact opposite nature of testosterone is a clear indication of medicine's lack of intellectual and clinical curiosity in the face of a highly politicized and rhetoric laden class of drugs. This lack of rigor and adherence to scientific principles persisted and researchers made conclusions which were erroneous, flawed, and simply wrong. This could not have been better displayed than by researchers studying AAS in hemodialysis patients.

Navarro JF et al. (1998) concluded androgen administration had beneficial effects on erythropoiesis, as well as positive anabolic actions in patients under peritoneal dialysis. [[46]] Gascon A et al. (1999) concluded, "The use of Nandrolone decanoate will allow us an acceptable treatment of anemia, as well as a better nutritional condition in elderly patients on dialysis." [[47]] Lastly, Johansen KL et al. (1999) concluded, "Treatment with Nandrolone for six months resulted in a significant increase in lean body mass associated with functional improvement in patients undergoing dialysis." If you look at testosterone and luteinizing hormone values at baseline, each decreased significantly at three months. [[48]] None of these published studies noted or referenced the previous work cited above that studied AAS in hemodialysis patients.

ADVERSE EVENTS

In the paper by Pena et al. many of the adverse events associated with ASIH are displayed. But even more remarkable is that the ignorance and unfamiliarity with AAS is there for all to see in a Board certified endocrinologist and urologist.

The patient was an HIV+ married male discovered to be azoospermic when the couple was exploring artificial insemination as an option to have children. His medications included testosterone enanthate and oxandrolone. To restore spermatogenesis the urologist discontinued only the testosterone and allowed the patient to remain on oxandrolone. Within months of this action the patient's testosterone level was 30 nanograms per deciliter, with luteinizing hormone (LH) and follicle-stimulating hormone (FSH) both below normal range, and suffering from notable depression and irritability that necessitated antidepressant medication. A repeat semen analysis continued to demonstrate azoospermia.

At this point the patient was referred to a medical endocrinologist for the evaluation of central hypogonadism. Pituitary and thyroid disorders were ruled out by normal serum prolactin and thyroid hormone levels, respectively. Magnetic resonance imaging of the brain and pituitary were normal. Finally, a decision was made that the patient's continued hypogonadism after testosterone cessation was due to the oxandrolone. After discontinuing both testosterone enanthate and oxandrolone for three months the patient's serum testosterone rose to 134ng/dL which was sufficient for the production of spermatozoa. The patient was then encouraged to restart his androgen supplementation to improve both physical and emotional well-being.[[49]]

The evaluation and management of this patient was extraordinarily poor and inept. First, it is incredulous that these physicians are apparently unfamiliar with oxandrolone. Despite this they continued to treat him and order test which are costly and unnecessary. The MRI was without any medical indication, particularly in the face of the known medications testosterone and oxandrolone. It is fortuitous that the MRI was negative since ~10% of the general populations have asymptomatic pituitary adenomas.

This patient demonstrates the pervasive effect upon the health and welfare those AAS studies which failed to account for homeostasis. Clinicians across the USA and beyond are using these studies as a basis for the clinical care of patients. That neither of these physicians even knew the most rudimentary AAS knowledge and was unaware as to ASIH after AAS cessation is horrific and shocking. But what is particularly disheartening is no one displayed any sense on what to do regarding the patient's HPTA. There are literally tens of thousands of patients in the United States who are receiving similar androgen treatment as the patient in Pena et al., each is potentially being left in the state of HPTA dysfunction.

LONG-TERM EFFECTS

Urhausen et al. (2003) studied serum parameters in 15 AAS users. The mean time after steroid cessation was 43 months with the minimum length of time 1 year and the maximum 10 years in the study. The average amount of medication used was a mean of 700 milligrams for 26 weeks, half a year, for 9 years. [[50]] The long-term side-effects of anabolic steroid use were demonstrated to be most pronounced on the HPTA. It was found A13/15 ex-AAS users were found in the lower 20 percent of the normal reference range for testosterone, 2/15 ex-AAS users were found below the normal range with values of 6.6 and 9.0 nanomoles per liter.

vanBreda et al. (2003) presents a case study in a 37y male who after AAS cessation had persistent HPTA dysfunction. [[51]] Restoration of HPTA dysfunction was achieved with the use of LH-RH.

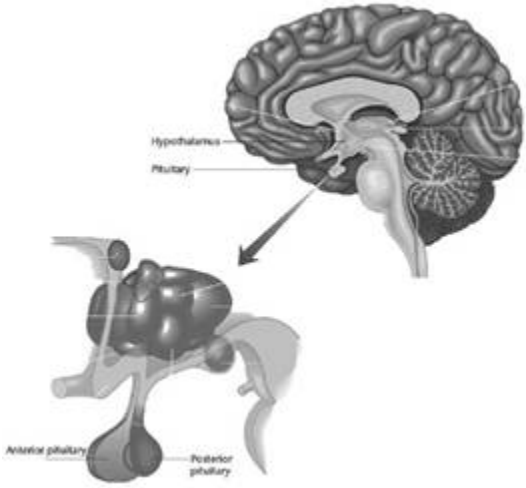
DURABILITY

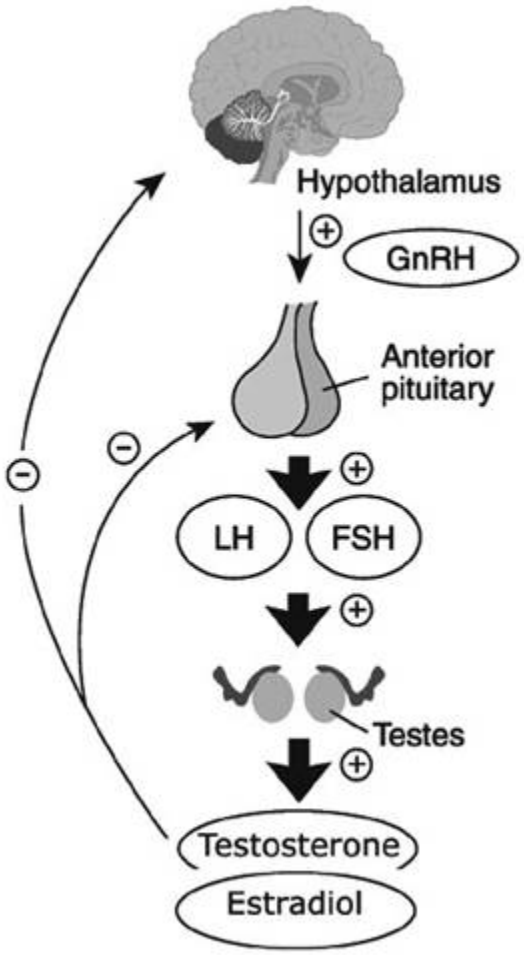
In 2004, Schroeder et al. included an equivalent amount of time for follow-up after AAS cessation as AAS administration. The study found that the positive body composition changes produced by the androgen in the study had completely disappeared after cessation. This was due to the state of hypogonadism induced by the administration of androgens (ASIH). Anabolic improvements were lost 12 weeks after discontinuing the androgen.[[52]]

The publication and timing of the study by Schroeder et al. is strongly suspect. This study may have never possibly been done if not for a formal complaint filed against the researchers through the Office of Human Research Protection (OHRP).[[53]] Also, documents received from researchers through the Freedom of Information Act (FOIA) conflict with data observed in the published study. Over 200+ pages were clearly missing in the materials sent.

HPTA NORMALIZATION & RESTORATION

TREATMENT





Autonomy.

There are vast differences between the health of an individual with frank hypogonadism (primary hypogonadism or testicular failure; secondary hypogonadism – hemochromatosis, Kleinfelters, etc.) and the individual with Andropause or PADAM (Partial Androgen Deficiency in Aging Male). The morbidity observed with true hypogonadism have been documented. While there are clinical indicators that are improved with AAS administration in Andropause there are no studies to show that these are factors for increased morbidity or an overall decreased quality of life. Until these studies are done care should be taken regarding the continuous long term administration of AAS.

There are also clinical situations which would necessitate AAS cessation for health concerns. With increasing AAS use these clinical conditions are sure to become increasingly prevalent. Compliance in taking medication is not 100% for a number of

reasons. This would lead to ASIH and potentially adverse events. A clinical situation would be elevation of LFTs (liver function tests) and impending liver dysfunction. Pens et. al. was a clear example of the adverse consequences with AAS cessation. AAS cessation was required in the treatment of polycythemia brought upon by continuous AAS administration.[]

A medical quandary for many physicians presented with hypogonadal patients, standard treatment to this point has been testosterone replacement therapy, human chorionic gonadotropin (hCG), or conservative therapy (i.e. nothing). The primary drawback of testosterone replacement is that this therapy is infinite in nature. Exogenous testosterone serves only to remedy the symptoms of suppressed testicular/gonadotropin production. While it may transiently combat the lean muscle atrophy, declining muscular strength, decreased libido, erection dysfunction, and depression associated with hypogonadism, it will not stimulate endogenous testosterone production. Administered testosterone will only suppress testicular function further.

It is important to understand that the use of a treatment for HPTA restoration at this time would only be effective in those individuals who had a normal HPTA functionality prior to AAS administration. This is not to say that there may be developed something in the future that will be effective for other causes of HPTA dysfunction. The regulation of the HPTA is an active area of investigation. There are other factors that interact with the HPTA which may show promise in their ability to restore HPTA health. The influences of other hormones within the endocrine system and the HPTA have only partially been explored.

The normal operation of both the testicular and hypothalamic-pituitary regions is crucial in returning HPTA function to normal. Returning one component of the axis to normal without concurrently returning the other would sabotage and inhibit the operation of the entire HPTA. The ability to produce a cure whereby there is no longer a need for medication is small. Discounting costs and focusing strictly on medicine reasons for this include inadequate stimulation for a critical part of the HPTA for full restoration, secondary inhibition of the HPTA, inadequate follow up and monitoring, and compliance due to the length of time the medicines are prescribed.

HISTORY

History has not been kind to AAS users whether illicit or prescribed. Undoubtedly, heavy politicization of AAS, constant media and press coverage, and the total failure of the medical community to properly investigate this class of medications have lead to ignorance among the public and professional, alike. The hysteria surrounding AAS is unprecedented as demonstrated by the draconian measures the government has applied to illicit AAS users. A considerable amount of the fault lies at the door of the medical profession who has capitulated lock, stock, and barrel to the pundits who barely are able to pronounce AAS never mind name on other than testosterone.

But what is the most horrific in the history of AAS is the mind warp that the medical/ research establishment took after 1995. While finally admitting that there is a positive

relationship between androgens and muscle the medical community has managed at the same time to have sentenced countless individuals to harm. One would have to been blind, deaf, and dumb and possibly dead to not recognize the relationship between androgens ands muscle. Apparently, the medical community was in a coma. The observational idea from association between androgens and muscle, of course, came from bodybuilders. Had any of the "white coats" ever come down from their Ivory Towers and bothered to ask the bodybuilders they would have been told about ASIH. It would not have been called that but there is no doubt they would have been told of post cycle signs and symptoms. But they did not ask, decided to ignore the principles of life, and in turn revealed once again their ability to make mistakes on a grand scale. Below is a summary of AAS history and the beliefs held by the athletic and bodybuilder community, academic/ physician, and what research ahs shown.

Beliefs Held by the Athletic and the Academic Communities On AAS		
BodyBuilders Beliefs held by recreational bodybuilders and athletic community.	**Research** What has been demonstrated..	**Physicians/Academics** The physician/ academic view and belief.
AAS increase muscle mass, strength, and athletic performance.	Replacement doses of testosterone when administered to hypogonadal men and supraphysiological doses when administered to eugonadal men increase fat-free mass, muscle size, and strength.	Only replacement doses of testosterone when given to hypogonadal men and prepubertal boys have anabolic effects. Supra-physiological doses of testosterone do not further increase muscle mass.
Higher doses of AAS promote greater increases in muscle mass and strength than lower doses; administering more than one androgenic steroid simultaneously (stacking) produces greater increases in muscle mass and	A linear dose–response relationship exists between testosterone dose and its anabolic effects over a wide range of concentrations extending from subphysiologic to supraphysiologic range.	Beyond the physiologic range, further increases in the dose of AAS would produce no further gains in fat-free mass and muscle strength.

strength than any single agent alone.		
The anabolic and androgenic activities of AAS can be dissociated, so that some derivatives of testosterone have preferentially greater anabolic activity than androgenic activity.	Different androgen-dependent processes have different dose– response relationships.	The anabolic and androgenic activity cannot be dissociated; they are described by the same dose–response relationship.
The anabolic and androgenic effects are mediated through separate mechanisms and thus can be dissociated.	The anabolic effects are likely mediated through an androgen-receptor-mediated mechanism that involves recruitment of tissue- specific coactivators and corepressors.	The anabolic effects are mediated through an androgen-receptor-mediated mechanism.
The effects of AAS administration cause an up-regulation of the skeletal muscle androgen receptor (AR).	AAS administration causes a upregulation of the skeletal muscle and bone androgen receptor (AR).	The effects of AAS administration cause a down-regulation of the skeletal muscle androgen receptor (AR).
HPTA Normalization after AAS cessation is variable and sometimes may never occur.	The severity and duration of ASIH after AAS cessation is unknown and has been reported to take over 2+years.	AAS cessation uniformly results in HPTA normalization within 2 weeks to several months.
Signs & symptoms after AAS cessation are due to inadequate gonadal function.	There is no medical or scientific literature that supports AAS dependency/ addiction. AAS dependency/addiction is not a recognized disease within the ICD-10 or the DSM-IV.	AAS use is associated with adverse health consequences that include chemical dependency/addiction.

FUTURE DIRECTIONS

It is time for the medical community to act responsibly, intelligently, and forcefully and take control of the medical care for individuals. At the very minimum the

I. Uniform definition and diagnosis of ASIH.

II. Investigations on a more accurate estimate of ASIH prevalence.

III. A does-response study on AAS and HPTA normalization. Clinical investigations regarding AAS (type, dose, duration, etc) to development of ASIH (severity of signs & symptoms, duration, HPTA normalization).

IV. Clinical investigations on medical treatments (prevent, eliminate, or minimize) for ASIH.

V. Investigations on the development of protocols or programs to effect positive body composition changes without the attendant consequences of ASIH.

VI. Collaborative clinical investigations regarding dependence, abuse, and addiction of androgens in relation to ASIH.

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PCT - An Uncontrolled Clinical Trial for Treatment of Androgen Induced Hypogonadism

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Objective: Although shown to be effective for their intended medical treatment, AAS have been shown to induce hypogonadotropic hypogonadism in adult males. The medical literature is conflicting in the reports of spontaneous return and long-term suppression of gonadal suppression post AAS usage. This observational study documents the treatment protocol of HCG, clomiphene citrate, and tamoxifen in returning hormonal function to normal post AAS usage. Design: Five HIV-negative males age 27-49, weighing 77-100 kg, with serum total testosterone levels below 240 ng/dL and luteinizing hormone (LH) levels below 1.5 mIU/mL were considered for this observational study. All five patients were administered the treatment protocol.

Methods: Treatment consisted of combination therapy which included concurrent administration of (a) Human Chorionic Gonadotropin, (b) Clomiphene Citrate and (c) Tamoxifen Citrate for a standard duration of 45 days. This protocol was repeated with every patient until serum LH and total testosterone values reached normal ranges.

Results: All five patients were considered eugonadal by normal laboratory reference ranges by the conclusion of treatment. Average serum total testosterone rose from 98.2 to 692.8 ng/dL ($p<.001$) while the average serum LH rose from an average undetectable value of less than 1.0 to 7.92 mIU/mL ($p<.0008$).

Conclusions: Although the treatment protocol of HCG, clomiphene citrate, and tamoxifen proved beneficial in reversing AAS induced hypogonadotropic hypogonadism, future controlled studies

need to be performed to confirm the beneficial effects of this combined pharmacotherapy in returning HPGA functioning to normal.

INTRODUCTION

Testosterone and testosterone analogues, anabolic-androgenic steroids (AAS), have long been used in the athletic community for improving lean muscle tissue and strength. A positive correlation has been shown with testosterone to include: increased protein synthesis resulting in lean muscle tissue development (Bhasin et al, 1996; 1997; Hervey et al, 1981; Tenover, 1992), enhanced sexual desire (libido) (Schiavi et al, 1991), increased muscular strength (Bhasin et al, 1996; 1997; Hervey et al, 1981; Sih et al, 1997), increased erythropoiesis (Bhasin et al, 1997; Evans & Amerson, 1974; Sih et al, 1997; Tenover, 1992), a possible positive effect on bone development (Anderson et al, 1996; 1997; Baran et al, 1978; Tenover, 1992), improved mental cognition and verbal fluency (Alexander et al, 1998), and male masculinizing characteristics (Starr & Taggart, 1992). Recently, however, clinicians have recognized the potential benefits of their use in the treatment of various disorders and ailments. Numerous studies have discussed the use of AAS in the treatment of HIV-associated conditions (Bhasin et al, 2000; Grinspoon et al, 1998; 1999; 2000; Rabkin et al, 1999; 2000; Sattler et al, 1999; Strawford et al, 1999; Van Loan et al, 1999), hypogonadism (Bhasin et al, 1997; Davidson et al, 1979; Rabkin et al, 1999; Sih et al, 1997; Snyder et al, 2000; Tenover, 1992; Wagner & Rabkin, 1998; Wang et al, 2000), impotence (Carani et al, 1990; Carey et al, 1988; Klepsch et al, 1982; Lawrence et al, 1998; McClure et al, 1991; Morales et al, 1994; 1997; Nankin et al, 1986 Rakic et al, 1997; Schiavi et al, 1997), burn victims (Demling et al, 1997), various anemia's (Doney et al, 1992; Gascon et al, 1999; Hurtado et al, 1993; Stricker et al, 1984), deteriorated myocardium (Tomoda, 1999), glucose uptake (Hobbs et al, 1996), continuous ambulatory peritoneal dialysis (CAPD) (Dombros et al, 1994), alcoholic hepatitis (Bonkovskyet al 1991; Mendenhall et al, 1993), hemochromatosis (Kley et al, 1992) and prevention of osteoporosis (Anderson et al, 1996; 1997; Baran et al, 1978; Behre et al 1997; Hamdy et al, 1998; Prakasam et al, 1999).

While AAS have proven effective in cases of lean muscle wasting conditions (HIV/AIDS), this class of medicines is not without their inherent problems. AAS have been shown to induce hypogonadotropic hypogonadism (Alen et al, 1987; Bhasin et al, 1996; Bijlsma et al, 1982; Clerico et al, 1981; Jarow & Lipshultz, 1990; Strawford et al, 1999; Stromme et al, 1974). This condition typically results from an abnormality in the normal functioning of the hypothalamic-pituitary-gonadal axis (HPGA), usually from a negative feedback inhibition of one of the hormone secreting glands, causing a cascading unbalance in the rest of the axis. Possibly resulting from a physiological abnormality (i.e. mumps orchitis, Klinefelters syndrome, pituitary tumor) or as an acquired result of exogenous factors (i.e. androgen therapy, AAS administration). Clerico et al (1981) found a dramatic suppression of serum gonadotropin levels in athletes given methandrostenelone, suggesting a direct action of AAS on the hypothalamus. Similar results of suppressed gonadotropins have been found in patients supplementing solely testosterone (Bhasin et al, 1996; Marynick et al, 1979; Strawford et al, 1999; Tenover, 1992). Case report studies discussed a 36-year old male competitive bodybuilder and a 39-year old father, each using various AAS regimens over extended periods of time, who showed a blunted response to GnRH stimulation tests (Jarow & Lipshultz, 1990). One particular study administered 600 mg of nandrolone decanoate to 30 HIV-positive males over twelve weeks (Sattler et al, 1999). The results made no reference to LH or testosterone levels. The lack of gonadotropin measurement is

puzzling as the data showed 12 of 30 subjects experienced testicular shrinkage, implying Leydig cell dysfunction and suppressed testosterone levels. Other studies using AAS have also shown no reference to LH or FSH levels but suppressed values are expected in each case (Bagatell et al, 1994; Behre et al, 1997; Sheffield-Moore et al, 1999; Tricker et al, 1996).

Declining, or suppressed, circulating testosterone levels as a result of either pathophysiological or induced hypogonadal conditions can have many negative consequences in males. Declining levels of testosterone have been directly linked to a progressive decrease in muscle mass (Mauras et al, 1998), loss of libido (Schiavi et al, 1991), decrease in muscular strength (Balagopal et al, 1997; Mauras et al, 1998) impotence (Rakic et al, 1997), oligospermia or azoospermia (Vermeulen & Kaufman, 1995), increase in adiposity (Mauras et al, 1998) and an increased risk of osteoporosis (Wishart et al, 1995). While some research suggests that the hormonal axis will spontaneously return to normal shortly after cessation of testosterone administration (Knuth et al, 1989), documented cases have taken up to 2 ½ years to return to normal (Jarow & Lipshultz, 1990). This case of a 39-year old male who previously used AAS was found to have low serum testosterone levels (6nmol/L, range 14 to 28 nmol/L) 2 ½ years after his last administration of the drugs (Jarow & Lipshultz, 1990). For most men, suffering with diminished libido, impotence, depression, fatigue, muscle atrophy, and infertility for 2 ½ years is not a pleasant option. Other androgen or anabolic steroid induced cases of hypogonadotropic hypogonadism have taken 6 months (Gazvani et al, 1997; Wu et al, 1996), 8 months (Gazvani et al, 1997), 10 months (Boyadjiev et al, 2000), 12 months (Schurmeyer et al, 1984), and 18 months (Gazvani et al, 1997) to finally return to eugonadal status.

The individual use of human chorionic gonadotropin (HCG), clomiphene citrate, and tamoxifen citrate in the treatment of testicular sub-function and gonadotropin suppression, respectively, is well documented. HCG has been shown to significantly improve gonadal function in hypogonadotropic hypogonadal adult males (Barrio et al, 1999; Burgess & Calderon, 1997; Cisternino et al, 1998; D'Agata et al, 1982; 1984; Dunkel et al, 1985; Kelly et al, 1982; Ley & Leonard, 1985; Liu et al, 1988; Martikainen et al, 1986; Okuyama et al, 1986; Ulloa-Aguirre et al, 1985; Vicari et al, 1992). Studies using clomiphene citrate to induce endogenous gonadotropin production in males found significant improvements in LH and FSH values after treatment (Bjork et al, 1977; Burge et al, 1997; Guay et al, 1995; Landefeld et al, 1983; Lim & Fang, 1976; Ross et al, 1980; Spijkstra et al, 1988). Tamoxifen citrate has also been found to produce a profound increase in serum LH levels as well as improved semen and sperm

quality (Gazvani et al, 1997; Krause et al, 1985; Lewis-Jones et al, 1987; Wu et al, 1996). As HCG's effect is centralized at the Leydig cells of the testicles, clomiphene citrate and tamoxifen citrate act upon the hypothalamic-pituitary region in stimulating gonadotropin production. Tamoxifen, a nonsteroidal antiestrogen, and clomiphene citrate, a nonsteroidal ovulatory stimulant, compete with estrogen for estrogen receptor binding sites, thus eliminating excess estrogen circulation at the level of the hypothalamus and pituitary and allowing gonadotropin production to resume normally. The normal operation of both the testicular and hypothalamic-pituitary regions is crucial in returning HPGA function to normal. Returning one component of the axis to normal without concurrently returning the other would sabotage and inhibit the operation of the entire HPGaxis. It was with this understanding that HCG was eventually combined with clomiphene citrate and tamoxifen as attempted therapy to reverse gonadal

function in hypogonadotropic hypogonadal males. In accordance with previous studies, each medication was used individually, and along with HCG, in initial trials. The simultaneous use of clomiphene citrate and tamoxifen was determined through preliminary use of clomiphene citrate and tamoxifen individually**. It was discovered that although both clomiphene citrate and tamoxifen met with some success, when combined together they achieved a more significant increase in gonadotropin production. This clinical outcome resulted in the combination therapy of HCG, clomiphene citrate and tamoxifen.**

Following is a clinical evaluation of the combined, simultaneous use of HCG, clomiphene citrate, and tamoxifen citrate as a treatment option in suppressed testosterone and gonadotropin levels in hypogonadotropic hypogonadal adult males. This observational analysis of the aforementioned treatment protocol assessed the efficacy of these medicines under non-controlled conditions.

METHODS

An observational study was done on the medical records of 5 adult male patients presenting to a clinic with induced hypogonadotropic hypogonadism. Patients were monitored and treatment recorded for the purposes of this observational study.

SUBJECTS

The medical records of five males age 27-49, mean 35.2, weighing 77-100 kg, mean 89.8 kg, with serum total testosterone levels below 240 ng/dL and serum luteinizing hormone (LH) levels below 1.5 mIU/mL were examined. Average presenting testosterone level was 98.2 ng/dL (normal= 240-827 ng/dL) while average LH level was undetectable at <1.0 mIU/mL (normal= 1.5-9.3 mIU/mL). The 5 patients had a history of AAS usage ranging from 9-60 months prior to presentation. All patients had ceased any testosterone therapy or AAS usage prior to initiation of treatment. Initial laboratory values confirmed that all patients had discontinued AAS long enough for endogenous lab values to fall below normal reference ranges. All patients were muscular in nature with an average BMI less than 27 at presentation. Table 1 presents the patient characteristics, anabolic history, and side effects upon presentation of the 5 patients.

LABORATORY STUDIES

Initial blood screening consisted of: AST, ALT, GGT, TOTAL CHOLESTEROL, LH, FSH, TESTOSTERONE, GLUCOSE, PROLACTIN, PSA TOTAL, TSH, T3 UPTAKE, T4 TOTAL, T4 FREE, HEMOGLOBIN, HEMATOCRIT

Table 2 shows all baseline serum blood levels at presentation. Baseline blood screening excluded any form of hyperprolactinemia or hypothyroidism as causes of hypogonadism in most patients. After physician examination and history and physical evaluation, it was determined that a history of AAS usage was present and most likely the cause of the patients' hypogonadotropic hypogonadal lab values; not hyperprolactinemia or hypothyroidism. Laboratory testing was performed by Quest Diagnostics Inc., (Houston, TX) and SmithKline Beecham Clinical

Laboratories, (Houston, TX). Repeat serum LH & testosterone samples were measured by immunoassay using chiron reagant kits on an ACS-180 instrument.

METHODS

A review of patients' medical records showed a treatment intervention of (a) human chorionic gonadotropin (HCG) (Ferring Pharmaceuticals), (b) clomiphene citrate (Teva Pharmaceuticals), and (c) tamoxifen (AstraZeneca). Typical dosage of HCG consisted of 2500 units every other day for 16 days. All HCG injections were self-administered intramuscularly. Starting dosages of clomiphene citrate and tamoxifen were 50mg and 20 mg daily, respectively. Patients started all three medications simultaneously and reported for the first follow-up blood work after completion of HCG, 16 days later. The post HCG blood analysis assessed testosterone-total response only. If testicular stimulation, i.e. testosterone production, was inadequate, additional HCG was administered at this stage of therapy rather than waiting an additional 30-45 days before the protocol completion. If the testicular response to the HCG demonstrated sufficient testicular stimulation (typically a blood serum level of >300 ng/dL), clomiphene citrate and tamoxifen were continued for 15 and 30 days, respectively. The arbitrary cut-off level of 300 ng/dL was used as a general assessment where sufficient Leydig cell stimulation was taking place even in light of artificial stimulation from HCG. A repeat blood sample was then taken at day 45 to assess hypothalamic-pituitary-gonadal axis status via luteinizing hormone and total testosterone levels. Because of the varying cessation times of the medications, the concluding blood sample was taken after a 30 and 15-day washout period of HCG and clomiphene citrate, respectively. For HPGA function to be considered normal, both LH and testosterone values had to fall within the normal reference ranges. For the purposes of patient treatment, if LH and testosterone values were still below normal limits at the conclusion of 45 days of treatment, a repeat protocol administration of HCG, clomiphene citrate, and tamoxifen was given. This protocol was repeated with every patient until LH and testosterone values reached normal ranges.

RESULTS

All five patients were considered eugonadal by normal laboratory reference ranges by the conclusion of treatment. Average serum total testosterone rose from 98.2 to 692.8 ng/dL. Average serum LH rose from <1.0 to 7.92 mIU/mL. An average of 48,974 U of HCG (five 10,000 Unit boxes), 3412.5 mg of clomiphene citrate (68.25 50mg tablets), and 968.71 mg of tamoxifen (48.44 20mg tablets) were used to treat all patients to eugonadal. Total treatment time ranged from 43-120 days. Mean elapsed time from initiation of treatment to eugonadal was 68.6 days. Statistical analysis was performed using repeated measures ANOVA. Pre and post treatment testosterone values were significantly ($p<.001$) different as were the LH values ($p<.0008$). Table 3 demonstrates the hormone changes during the treatment period and the duration to eugonadal.

ADVERSE EVENTS

None of the study subjects had any serious or treatment-terminating effects as a result of the multi-drug protocol. No problems were noted with regards to parameters of normal urologic function or treatment causing gynecomastia. Any side effects documented at presentation were

reversed by the conclusion of treatment.

DISCUSSION

This observational study demonstrates the possible efficacy of HCG, clomiphene citrate, and tamoxifen citrate in returning the HPGA to normal physiological function in adult males suffering from androgen induced hypogonadotropic hypogonadism. In the case of decreased testicular function manifested by low testosterone levels, it is of primary importance to first return the normal function of the testicular cells. The initial lack of response to HCG should not immediately be a cause for the initiation of testosterone replacement therapy, as with the current accepted therapy modality by many physicians. Blood analysis confirmed that no exogenous testosterone was administered during the treatment period, as exogenous androgens would have had a suppressive effect on endogenous gonadotropin production. Therefore, because of the corresponding normal gonadotropin and testosterone values, it is accepted that gonadotropin and testicular function were normal by the conclusion of treatment.

The standard treatment of HIV-related muscle wasting, AAS therapy, may involve decades of treatment and the attendant problems with any therapy of a prolonged nature. Polycythemia vera, elevated hepatic enzymes, and prolonged negative alterations in lipid profile are a few of the dangers experienced by HIV patients administered AAS for extended periods. Of greatest concern is the increasing numbers of individuals who are currently being treated with AAS to increase muscle mass either for medicinal or recreational means without attention being given to periodically returning the HPGA to normal. With roughly 4 million men in the U.S. being considered hypogonadal (Lacayo R., 2000; Sheffield-Moore et al, 1999; Shelton DL, 2000), an estimated 200,000 men are currently receiving testosterone treatment for the condition (Shelton DL, 2000). As stated earlier, AAS are being prescribed to HIV & AIDS sufferers to combat progressive muscle loss. The Centers for Disease Control and Prevention (CDC) reported an estimated 635,000+ men diagnosed with AIDS through December 2000 while an estimated 97,700 have been reported with HIV (Centers for Disease Control, vol.12, No. 2, table 5; Centers for Disease Control, vol. 12, No. 2, table 6). In 2000 alone over 31,000 men were diagnosed with the AIDS virus (Centers for Disease Control, vol. 12, No. 2, figure 3). Between hypogonadal, AIDS, & HIV males, potentially over 900,000 men are being administered AAS therapy.

Studies recently published on patients suffering from various tissuedepleting conditions and HIV affliction (Bhasin et al, 2000; Grinspoon et al, 1998; 1999; 2000; Rabkin et al, 1999; 2000; Sattler et al, 1999; Strawford et al, 1999;1999; Van Loan et al, 1999) have not identified what should be done to restore normal endocrine status post-treatment. Considering the dosages and compounds administered in many studies, there is no question that subjects were left hypogonadal after therapy. In the cases where the periodic use of testosterone or AAS are necessary, intervention to return the HPGA to normal should be initiated as soon as possible after the cessation of the AAS. As described herein, a possible treatment modality may be the combined regimen of HCG, clomiphene citrate, and tamoxifen.

Medical history has demonstrated examples of physician-induced complications resulting from treatment. Iatrogenic hyperthyroidism (Bartsch & Scheiber, 1981) and iatrogenic Cushing's syndrome (Cihak & Beary, 1977; Kimmerle & Rolla, 1985; Smidt & Johnston, 1975; Tuel et al,

1990) are cases were administered medications or treatments provoked abnormalities in patients' normal physiology. The administration of testosterone as a treatment for hypogonadotropic hypogonadism falls into this same category of causing endocrine related abnormalities (Bhasin et al, 1996; Marynick et al, 1979; Strawford et al, 1999; Tenover, 1992). Testosterone replacement therapy has proven to be very effective in reversing the symptoms of suppressed testosterone production, but does not treat the underlying cause of the deficiency. Positive effects of testosterone treatment; i.e. improved sex drive, improved sense of well-being, lean body mass; are all transient in light of plummeting gonadotropin levels. Upon cessation of testosterone treatment patients can expect a complete reversal of positive benefits as exogenously influenced testosterone levels metabolize and decline rapidly. Further controlled studies need to be performed showing the combined effects of HCG, clomiphene citrate, and tamoxifen in returning HPGA functioning to normal. Long-term follow-up on these patients returning to normal will be necessary to ensure permanent reversal of hypogonadotropic hypogonadal conditions. In addition, studies documenting dose-response curves for pituitary inhibition and reversal due to AAS administration are critical in determining the correct dose, duration, and form of treatment that is optimal without causing permanent damage. When the need for long-term androgen use presents, using moderately supraphysiologic doses of androgens as suggested by Strawford and colleagues (1999) coupled with post-treatment HPGA restoration as demonstrated here, may be a more effective means over high-dose protocols used to offset negative alterations in lean body mass. Unfortunately current studies have yet to adequately address a standard of patient care post-androgen therapy. Because of the negative impact of the hypogonadal state on physical and mental well- being, pharmacotherapy that restores HPGA function more rapidly than current modalities would greatly benefit men with hypogonadotropic hypogonadism.

While we believe that the treatment protocol was effective in returning normal hormonal function to these men, the lack of randomization or a control group leaves room for speculation. Although cases of spontaneous return to eugonadism with no medicinal intervention have been published, these reports documented durations anywhere from 6-18 months before normal hormone status was achieved (Gazvani et al, 1997; Wu et al, 1996). If the alternative treatment modality described herein can reverse suppressed gonadotropin production and AAS associated side effects much sooner than non-treatment, further evaluation of this therapy should continue.

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> ABBREVIATIONS
AAS Anabolic-Androgenic Steroids
AIDS Acquired Immunodeficiency Virus
ALT Alanine aminotransferase
AST Aspartate aminotransferase
BMI Body Mass Index
dL deciliter
FSH Follicle Stimulating Hormone
GGT Gamma-glutamyl transferase
GnRH Gonadotropin Releasing Hormone
HCG Human Chorionic Gonadotropin
HIV Human Immunodeficiency Virus
HPGA Hypothalamic Pituitary Gonadal Axis
kg kilogram
LH Luteinizing Hormone
mg milligram
mIU mili International Units
mL milliliter
ng nanogram
PSA Prostate Specific Antigen
T3 Triiodothyronine
T4 Thyroxine
TSH Thyroid Stimulating Hormone

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CASE REPORT

Conservative management of azoospermia following steroid abuse

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As well as athletes and competitive body builders, recreational body builders attending gymnasia are known to abuse anabolic steroids, using doses from 10- to 40-fold above physiological levels. Androgenic steroids induce hypogonadotrophic hypogonadism with associated azoospermia, leading to infertility. Little literature exists on the treatment of steroid-induced azoospermia following the cessation of abuse. We present four cases of steroid-induced azoospermia, its conservative management and eventual return of normal semen density.

Key words: abuse/azoospermia/infertility/steroid

Introduction

Anabolic steroids have been used by athletes to improve strength and performance for more than 40 years (Lukas, 1993). Abuse by competitive body builders is thought to be common, but recreational body builders attending gymnasia also abuse steroids (Perry *et al*., 1992). In these settings, high doses from 10- to 40-fold above physiological levels are being used (Kilshaw *et al*., 1975; Knuth *et al*., 1989).

Among well-reported side-effects, androgenic steroids also induce hypogonadotrophic hypogonadism with associated azoospermia (Schurmeyer *et al*., 1984). Hence, users of anabolic steroids often seek medical attention because of infertility (Kilshaw *et al*., 1975). However, little literature exists on the treatment of steroid-induced infertility and the duration of azoospermia following the cessation of abuse.

We present four cases of steroid-induced azoospermia which returned to normal spontaneously, following cessation of steroid abuse, with pregnancies occurring in at least three of the cases. All patients were questioned in detail concerning the type, dosage and frequency of drug taking. However, it was apparent that the patients were reluctant to give a detailed account and it was impossible to be certain as to the accuracy of their histories.

Case 1

A 31 year old man was referred to the regional andrology clinic with a 6 year history of secondary infertility due to azoospermia. He was an amateur body builder and had been taking 8 week courses of steroids during the last 5 years. On examination testicular volume was found to be reduced to 15 ml. Seminal analysis confirmed azoospermia. Serum follicle stimulating hormone (FSH) values were 0.6 U/l (2.0–8.0) and luteinizing hormone (LH) values were 2.8 U/l (2.0–10.0). Serum testosterone concentrations were 3.7 nmol/l (9.0–40.0 nmol/l). The patient was advised to discontinue steroids.

One year following the cessation of steroid abuse there was some improvement in conventional seminal parameters. Sperm concentration was 17×10^6/ml, with 20% progressive motility and 25% flagellate motility. Similarly pituitary gonadotrophins: were FSH 2.1 U/l, LH 3.9 U/l and testosterone 7.3 nmol/l.

Eighteen months into the cessation of steroid abuse the sperm concentration was 112 $\times10^6$/ml with 47% progressive motility and 5% flagellate motility. The FSH was 2.6 U/l, LH 5.1 U/l and testosterone 6.6 nmol/l. The couple achieved a pregnancy spontaneously 20 months after cessation of steroid abuse.

Case 2

A 33 year old man was referred to the regional andrology unit for consideration for treatment with donor semen following a diagnosis of primary infertility due to azoospermia. He was a keen body builder and was using steroids. The testicular volume was reduced to 12 ml. His endocrine profile on presentation was FSH 0.6 U/l, LH 1.8 U/l, testosterone 24.6 nmol/l, and the semen analysis confirmed azoospermia.

He was counselled as to the avoidance of steroids and within the first 6 months of abstinence the sperm concentration was 7.4 $\times10^6$/ml with 35% progressive motility and 7% flagellate motility. His FSH was 1.9 U/l, LH 4.3 U/l and testosterone 1.3 nmol/l. Eight months into the conservative management the sperm concentration had improved to 32.9 $\times10^6$/ml, 56% progressive motility and 26% flagellate motility. The FSH was 2.3 U/l, LH 3.7 U/l, testosterone 11.2 nmol/l and the testicular volume 18 ml. Despite the improvement of semen parameters the patient did not attend any further clinic appointments. He was later found to have returned to steroid abuse.

Case 3

A couple (husband aged 27 years) was referred to the clinic with primary infertility of 3 years. Initial investigations had

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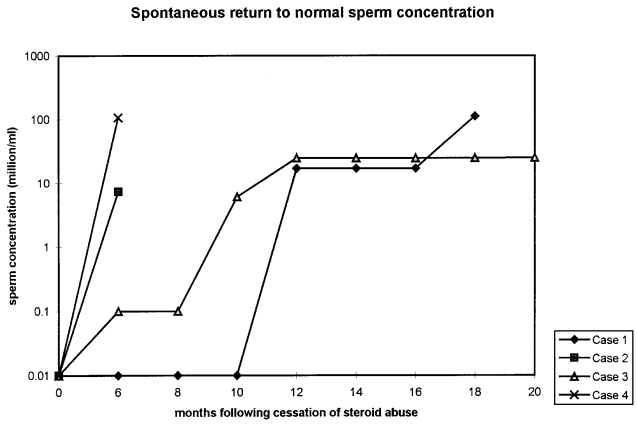

Figure 1. The spontaneous return of sperm concentration to normal values during abstinence from steroid abuse.

shown azoospermia. He was a body builder and had been abusing steroids. He was advised accordingly regarding the abuse of steroids. A repeat semen analysis after 6 months abstinence from steroids showed a sperm concentration of 1×10^5/ml with 30% progressive motility and 60% flagellate motility. He was then referred to the regional andrology clinic. On examination testicular volume was normal (20 ml), FSH was 1.9 U/l, LH 4.6 U/l. Testosterone values were not measured due to the small volume of blood available. Five months later the sperm concentration was 6.1×10^6/ml with 35% progressive motility and 30% flagellate motility. Thirteen months after the cessation of steroid use the sperm concentration was 24.8×10^6/ml with a progressive motility of 25% and flagellate motility of 15%. After 20 months of abstinence the sperm concentration was 25×10^6/ml with a 55% progressive motility and 16% flagellate motility. Two months later, 22 months after discontinuing steroids, the couple achieved a pregnancy.

Case 4

A 28 year old man was referred with 1 year of primary infertility due to azoospermia. He was a body builder and had been abusing steroids for over 4 years. On examination testicular volume was reduced to 14 ml. On the first investigation azoospermia was confirmed alongside suppressed gonadotrophin levels: FSH 0.7 U/l and LH 1.2 U/l. Testosterone concentration was 4.2 nmol/l. Five months after abstinence, his FSH was 0.9 U/l, LH 1.5 U/l, testosterone 6.1 nmol/l and sperm concentration 51.1×10^6/ml with 38% progressive motility and 48% flagellate motility. Testicular volume was normal (20 ml) at that time. A month later the FSH was 0.9 U/l, LH 1.5 U/l, testosterone 11.2 nmol/l, and sperm concentration 106×10^6/ml with 31% progressive motility and 50% flagellate motility. The couple achieved a pregnancy by the ninth month following the cessation of steroids.

The improvement in sperm concentration of the four cases is summarized in Figure 1.

Discussion

Steroid abuse by athletes has continued to increase despite the efforts of various sports organizations to curb a practice which had previously been restricted to weight lifters and professional body builders (Buckley *et al*., 1988). Recreational body builders attending gymnasia are also abusing steroids (Perry *et al*., 1992) but the frequency and patterns of use and the associated problems are less well known.

Spermatogenesis is under the control of FSH and LH, whose secretion is regulated by gonadal steroids and possibly inhibin. Administration of anabolic steroids, as derivatives of testosterone, suppresses gonadotrophin secretion. A hypogonadal state can be induced that is characterized by decreased serum gonadotrophins, decreased serum testosterone concentrations (unless exogenous testosterone is being administered at the time of testing), testicular atrophy and impaired spermatogenesis. These effects result from the negative feed-back of androgens on the hypothalamic–pituitary axis and possibly from local suppressive effects of excess androgens on the testis (Kilshaw *et al*., 1975; Holma and Aldercreutz, 1976; Jarow and Lipshultz, 1990).

The patterns of anabolic steroid administration are largely complex and without a standard. It is essentially difficult to obtain information and history from abusers and it is not always reliable. It is estimated that there are more than 45 anabolic steroid compounds available for abuse by athletes, and their use is increasing among male and female adolescents (Lane and Connor, 1994). Frequently oral and parenteral hormones are used in what is referred to as ‘stacking’ in an effort to maximize steroid receptor binding and increase the desired effects (Lukas, 1993). Steroids are usually cycled, or used for 6–12 weeks followed by abstinence for several weeks (Turek *et al*., 1995). Amongst the preparations used are testosterone esters (propionate, phenylpropionate, isocaproate, and decanoate), testosterone oenanthate, nandrolone decanoate, mestanolone (methylandrostanolone) oenthate in i.m. injectable form, and methandienone, oxandrolone and stanozolol in tablet form.

Androgenic steroids are known to cause impaired spermatogenesis (Heller *et al*., 1950) and the use of 19-nortestosterone has even been suggested for male contraception (Schurmeyer *et al*., 1984; Knuth *et al*., 1985). Hormonal suppression of sperm production provides reversible contraception (Wu, 1996). Infertility following anabolic steroid use commonly presents as oligozoospermia or azoospermia along with abnormalities of sperm motility and morphology (Kilshaw *et al*., 1975; Holma, 1977; Knuth *et al*., 1989; Jarow and Lipschultz, 1990). Data available from the development of androgenic steroids for male contraception indicate that reversal of effects can take up to 12 months after discontinuation of the drugs (Schurmeyer *et al*., 1984). However, the doses used by body builders may be up to 40-fold higher than therapeutic doses. Furthermore, the multiple preparations used makes it almost impossible to study the adverse effects of individual drugs (Lloyd *et al*., 1996).

There is little literature and considerable disagreement regarding the management of these cases and the treatment of prolonged azoospermia. In a retrospective, cross-sectional, case–control study (Knuth *et al*., 1989) it was suggested that even after prolonged use of high doses of anabolic steroids, sperm production returns to normal without any active treat-

ment. On the other hand Turek *et al*. (1995) suggested that if the semen parameters and endocrine balance are not restored 6 months after the cessation of steroid use the patients should be treated in a similar manner to that used for the Kallman syndrome, hypophysectomy or other forms of hypogonadotrophic hypogonadism. In these diseases there is a lack of FSH and LH production, and a low serum testosterone concentration similar to the findings in athletes taking anabolic steroids (Holma and Adlercreutz, 1976; Knuth *et al*., 1989). Treatment for virilization in these patients involves weekly doses of i.m. testosterone. However, the induction of spermatogenesis requires treatment with gonadotrophins or gonadotrophin analogues, including intramuscular injections of human chorionic gonadotrophin (HCG) (Martikainen *et al*., 1986; Burris *et al*., 1988; Jarow and Lipshultz, 1990) and human menopausal gonadotrophin (HMG) (Burris *et al*., 1988). Subsequently, spermatogenesis can be maintained by HCG injections alone (Turek *et al*., 1995).

The prolonged suppression of gonadotrophin secretion following steroid abuse is analogous with the prolonged suppression of thyroid stimulating hormone following excessive thyroxine intake and the suppression of ACTH from the adrenal following excess hydrocortisone (glucocorticosteroid) therapy. One could speculate that the hypothalamic controlling peptides not only release pituitary hormones but also have a trophic action on the target cells themselves. The suppression of gonadotrophin releasing hormone (GnRH) by the anabolic steroid abuse, according to this hypothesis, would lead to gonadotroph atrophy in the pituitary. The variable return of pituitary function would therefore depend on the rate of recovery of gonadotroph from the atrophic process. Relatively lower doses of steroids used for contraceptive purposes would not be expected to cause, or to cause less extensive, gonadotroph atrophy, which can explain the shorter recovery time for normal spermatogenesis to return following the cessation of use.

Following discontinuation of steroid abuse and return of normal pituitary function one would not expect the sperm concentration to start improving immediately. Once spermatogenesis is arrested it may take as long as 64 days for spermatozoa to appear in the seminiferous tubules (Hellere and Clermont, 1963) and the duration of transit through the ductular system requires a median of 12 days (range 1–21 days) (Rowley *et al*., 1970).

Patients 1, 3 and 4 achieved a pregnancy at 2, 8 and 4 months respectively, following the return of normal semen parameters (concentration $>20\times10^6$/ml). Variation in the time interval between normal spermatogenesis and conception may be due to subtle differences in both male and female fertility status. Similar differences can be observed in all couples and the diagnosis of subfertility is considered only after 12 months of unprotected, regular intercourse.

Conclusion

In conclusion, our cases demonstrate that there is a tremendous variability in the return of spermatogenesis which is impossible to predict from the number, type or duration of anabolic steroids taken. However, it is apparent that prolonged azoospermia following steroid abuse can be successfully managed by conservative means alone and return of fertility achieved.

The slow recovery of gonadotrophins predicts an eventually successful return of fertility. More aggressive treatment with exogenous gonadotrophins and testosterone may be considered if there is absence of improvement in the sperm parameters for longer than 24 months.

It is, however, of paramount importance that awareness of these side-effects is increased among young men who take anabolic steroids recreationally without knowing the potentially serious consequences.

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responsible for the urticarial skin eruption that was the presenting symptom. Further clinical evaluation showed that the lymphocytic vasculitis also was responsible for the polymyositis that had been identified on physical examination and confirmed by serum enzyme levels. The cause of polymyositis, an inflammatory process affecting symmetrical skeletal muscle groups, is identified only in some patients.[6] Causative agents include adverse reactions to ingestants, autoimmune diseases, malignant neoplasms, sarcoidosis,[7-9] and vasculitis.[10] Although our patient had well-documented sarcoidosis in the past, our clinical, serologic, and histologic evaluation failed to provide any evidence of reactivation of her previous disorder. Thus, to our knowledge, this is the first reported case of a lymphocytic vasculitis producing both polymyositis and urticarial skin lesions.

The differential diagnosis of a lymphocytic vasculitis includes autoimmune diseases, infections, malignant neoplasms, adverse reactions to ingestants, and inflammatory disorders of unknown origin (Table 2).[11-15] When the cause of lymphocytic vasculitis is known, the underlying cause should be removed or treated. Patients such as the one reported here with an unidentified etiologic factor frequently require treatment with anti-inflammatory medication, which may include corticosteroids, azathioprine, and cyclophosphamide. When these medications are used, the lymphocytic vasculitis is usually well controlled.

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Impotence Related to Anabolic Steroid Use in a Body Builder

Response to Clomiphene Citrate

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THE RECREATIONAL USE of anabolic steroids has become commonplace among athletes.[1,2] Exercise enthusiasts frequently subscribe to information from such sources as the "Underground Steroid Handbook"[3] and self-design illicit drug therapy, including the use of human chorionic gonadotropin (hCG), clomiphene citrate (Clomid), and tamoxifen citrate, to counter the side effects of gynecomastia and reduced testicular volume. Despite this apparent drug sophistication, not only can these persons have a psychological dependence on the anabolic steroids,[4,5] but hypogonadotropic hypogonadism that lasts for months[6,7] to years[8] may also develop.

The case presented here illustrates the degree of drug knowledge among body builders, the psychosocial dependence on these drugs, and the potential of clomiphene[9] in treating the disorder of pituitary-gonadal failure in such persons.

Report of a Case

The patient, a 29-year-old man, had impotence and decreased libido for a year. He is a college student and a competitive body builder who had used anabolic steroids for eight months (January to August 1992), alternating 16-week cycles of testosterone cypionate (Depo-Testosterone), 1,500 to 1,800 mg per week, and oxymetholone (Anadrol), 560 mg per week. After stopping the use of these drugs in August 1992, he was impotent with no spontaneous erections and had diminished libido. He completed a self-selected four-week trial of human chorionic gonadotropin (hCG) in September 1992 without any change in libido and no improvement in potency. The dose of hCG is unknown, and the patient denied any previous use of the drug. He was advised by colleagues to take a course of clomiphene or await the spontaneous return of sexual

(Bickelman C, Ferries L, Eaton RP: Impotence related to anabolic steroid use in a body builder—Response to clomiphene citrate. West J Med 1995; 162:158-160)

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ABBREVIATIONS USED IN TEXT

FSH = follicle-stimulating hormone
Gn-RH = gonadotropin-releasing hormone
hCG = human chorionic gonadotropin
LH = luteinizing hormone

function. He elected to wait for nine months, without success.

He sought endocrine consultation in July 1993, almost a full year after his last steroid dose, because of continued impotence and reduced libido. On examination he was robust, weighing 76 kg (168 lb), height 178 cm (5 ft 10 in), appearing healthy, and was heavily muscled. He had a reduced testicular volume of 10 ml on both sides and 2 cm of gynecomastia on both sides. A urine screening test for exogenous anabolic steroids was negative for 19 steroids or metabolites, including danazol, fluoxymesterone, methyltestosterone, 19-nortestosterone, oxymetholone, and stanozolol, as well as the diuretic probenecid. An adrenocorticotropic hormone-stimulation test showed a normal rise in the cortisol level from 360 to 830 nmol per liter (13 to 30 μg per dl). Magnetic resonance imaging with gadolinium enhancement revealed a normal pituitary gland. Serum gonadotropin and free testosterone levels were abnormal, however, as shown in Figure 1, with a follicle-stimulating hormone (FSH) level of 0.6 mIU per ml (1.6 to 17.8 mIU per ml), a luteinizing hormone (LH) level of 1.9 mIU per ml (1.4 to 11.1 mIU per ml), and a free testosterone level of 7.1 pg per ml (19.0 to 41.0 pg per ml).

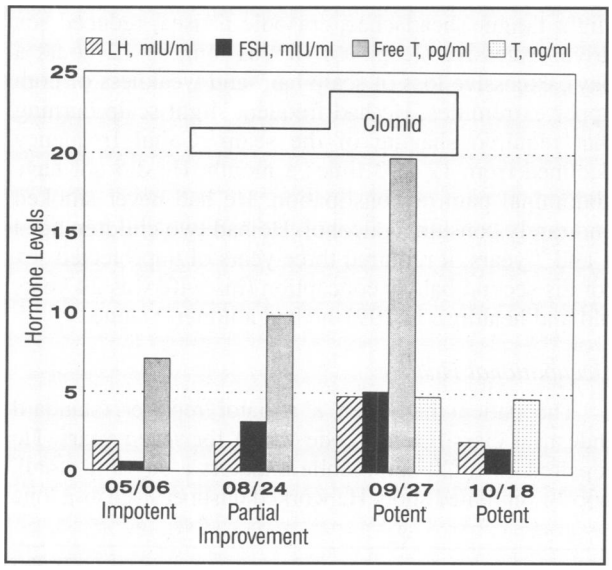

Figure 1.—The 5-month course of response to Clomid (clomiphene citrate) is represented by serial plasma levels of pituitary gonadotropins (luteinizing hormone [**LH**] and follicle-stimulating hormone [**FSH**]), gonadal free testosterone (**Free T**), and total testosterone (**T**). The associated improvement in sexual potency is seen to parallel the rise in free and total testosterone levels in response to Clomid therapy.

Treatment was initiated with clomiphene, 50 mg orally per day, and after a month of therapy he had noticed no improvement in potency or libido, although he had begun having morning erections. Serum hormone tests showed moderate improvement in FSH, LH, and free testosterone levels, although not in the normal range (Figure 1). A month after taking a double dose of clomiphene (100 mg per day), the patient reported an increase in libido and potency, and he was able to have sexual intercourse daily. His gonadal volume was unchanged, although serum FSH, LH, and free testosterone levels had reached normal for his age (Figure 1). After clomiphene therapy was discontinued three weeks later, the serum FSH and LH levels fell to normal, and the total serum testosterone remained at a normal level of 16.3 nmol per liter (4.7 ng per ml) (range, 12.5 to 34.5 nmol per liter [3.6 to 9.9 ng per ml]). This response suggested a restoration of normal hypothalamic-pituitary-gonadal function, and it was proposed to reevaluate this function with a longer follow-up to determine whether the correction was sustained.

Follow-up of the patient six months later revealed that he had returned to the illicit use of Depo-Testosterone at 400 mg per week to achieve a level of sexual performance three times that achieved with clomiphene alone. He noted that his testes were smaller, and he was considering trying another course of hCG in combination with tamoxifen to prevent worsening gynecomastia.

Discussion

The illicit use of anabolic steroids is becoming more widespread, especially among those involved in competitive athletics or body building and even among teenagers.[10] Even when gonadal dysfunction occurs, persons often continue using the anabolic steroids, in part because of the neuropsychiatric effects, which include psychotic symptoms, affective syndromes, increased aggression, and psychological dependence.[4,11] In lay literature, it is common to find medical discussions and advertisements concerning anabolic steroids, androgen supplements, and agents used to combat the side effects of gynecomastia, hirsutism, fluid retention, and acne (*MuscleMag International*, September 1994, pp 280-281).

Most synthetic anabolic steroids have some androgenic effects that inhibit gonadotropin-releasing hormone (GnRH) release from the hypothalamus and FSH and LH release from the anterior pituitary. This results in a hypogonadotropic state, and if the agents are used for a prolonged period, testicular atrophy with reduced serum testosterone levels results, causing reduced libido and impotence. When their use is discontinued, the feedback inhibition of GnRH, FSH, and LH synthesis and release is removed and the hypogonadotropic hypogonadism is expected to resolve. According to the literature reports,[5-7] this usually occurs within four months. Only two cases have been reported in which suppression of the hypothalamic-pituitary-testicular axis lasted

longer than four months.[7] The first of these patients was administered hCG, and the outcome was determined to be successful when his wife conceived. The second patient presented with decreased libido three years after his last use of anabolic steroid and was found to have a severely blunted response to a GnRH-stimulation test, consistent with hypothalamic-pituitary suppression.

The patient in the case reported here is unique not only in the year-long suppression of his hypothalamic-pituitary-gonadal axis, but also in the successful response to hypothalamic-pituitary stimulation with clomiphene. Although we do not know his gonadotropin and testosterone levels before he began using steroids, it is unlikely he had a preexisting GnRH-deficiency state (such as Kallmann's syndrome) as he had normal secondary sexual development of phallus and hair distribution before initiating exogenous steroid use. We assume that he was compliant in abstaining from exogenous steroids during the treatment period, based on the negative drug screen and compliance with clomiphene administration. More frequent, random screening would be needed to confirm this assumption. The self-administration of hCG should have elicited a testosterone response, but it is uncertain whether he received true hCG in adequate dosage. Because he perceived the failure of a self-initiated hCG trial, we opted for the use of clomiphene at dosages commonly used in women with hypothalamic-pituitary-ovarian failure. Clomiphene use has previously been reported for the treatment of men who, during evaluation for infertility, are found to have marginal testicular failure or poor gonadotropin production.[8] Clomiphene appears to produce an antiestrogen effect on the hypothalamus that results in increased GnRH release. In addition, clomiphene exerts an estrogenlike effect on the pituitary, increasing pituitary sensitivity to GnRH.[12]

We propose that with the use of clomiphene we were able to augment the hypothalamic and pituitary responses to his low but not absent ambient estrogen derived by aromatization from testosterone. This is the first reported case of clomiphene-induced restoration of FSH, LH, and free testosterone levels in a man with recreational steroid-induced pituitary-gonadal failure.

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Lead Poisoning in a Radiator Repairer

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MORE THAN 1.4 MILLION workers in the United States are at risk of exposure to lead in industries as diverse as radiator repair, construction, demolition, lead smelting, casting, foundry, and manufacture of stained glass or batteries.[1] Scientists have been aware of the toxic effects of lead since ancient times.[2-7] In 1978 this copious scientific knowledge led to the enactment of a comprehensive lead standard by the United States Occupational Safety and Health Administration (OSHA).[6] Unfortunately, compliance with this standard may be inadequate even today, as is exemplified by this urban case of lead poisoning in a radiator repairer.

Report of a Case

The patient, a 26-year-old man, was seen because of slight fatigue, headaches, episodic nausea, reduced sex drive, excessive sleepiness (for as long as 12 hours a day), excessive loss of scalp hair, and weakness of both upper extremities. He had frequent slight scalp burning that required shaving of the scalp. Coital frequency declined from 12 to 3 times a month. He did not have abdominal pain or constipation. He had never smoked and rarely consumed alcohol. He had two children aged 1 and 7 years; it required three years of unprotected sex for his second baby's conception (his wife was 24 years old and healthy). In 1991 he had a ureteral stone.

Occupational History

The patient had been a radiator repairer (standard industrial classification code 7539) for seven years. He repaired 15 to 20 automobile radiators a day in a small, poorly ventilated shop. He worked unsupervised as a lone

(Lohiya GS, Lohiya S: Lead poisoning in a radiator repairer. West J Med 1995; 162:160-164)

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Subcutaneous self-administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotrophic hypogonadism

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The efficacy and safety of highly purified follicle stimulating hormone (FSH) associated with human chorionic gonadotrophin (HCG) was studied in 60 men with hypogonadotrophic hypogonadism. Of these men, 16 suffered from Kallmann's syndrome, 19 from idiopathic hypogonadotrophic hypogonadism and 25 from hypopituitarism. Basal testosterone concentrations were found to be far below the normal range. At baseline, 26 patients were able to ejaculate and all of them showed azoospermia, while the remaining patients were aspermic. All patients self-administered s.c. injections of FSH (150 IU × three/week) and HCG (2500 IU × two/week) for at least 6 months and underwent periodic assessments of testicular function. Testosterone concentrations increased rapidly during treatment and all but one patient reached normal values. Testicular volume showed a sustained increase reaching almost 3-fold its baseline value. At the end of treatment, 48 patients (80.0%) had achieved a positive sperm count. The maximum sperm concentration during treatment was 24.5 ± 8.1 × 10^6/ml (mean ± SEM). The median time to induce spermatogenesis was 5 months. Eleven patients reported adverse events, generally not related to treatment. Three patients experienced gynaecomastia. No local reactions at injection site were observed. In conclusion, the s.c. self-administration of highly purified FSH + HCG was well tolerated and effective in stimulating spermatogenesis and steroidogenesis in these patients.

Key words: highly purified FSH/male hypogonadotrophic hypogonadism/spermatogenesis/subcutaneous self-administration/testicular size

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Introduction

Male hypogonadotrophic hypogonadism (HH) has been successfully treated for several decades by administration of gonadotrophins (Finkel *et al.*, 1985; Ley and Leonard, 1985; Okuyama *et al.*, 1986), which allows the restoration of testicular steroidogenesis and spermatogenesis. Human chorionic gonadotrophin (HCG) is normally used as the source of luteinizing hormone (LH) activity to stimulate testosterone secretion by Leydig cells, whereas human menopausal gonadotrophin (HMG) has been generally used as the follicle stimulating hormone (FSH) source to stimulate proliferation and maturation of germinal cells.

Since spermatogenesis is a time-consuming process, any attempt aimed at its restoration must rely on a long-term treatment; normally, thrice-weekly intramuscular (i.m.) HMG injections have been administered for several months. The i.m. administration involves various inconveniences, such as local pain or need to visit a health centre for injections, which are particularly relevant in the case of a chronic treatment, thus decreasing compliance and often leading to the interruption of treatment before spermatogenesis has been achieved (Saal *et al.*, 1991). Thus, the availability of a treatment which could be given s.c. is especially advantageous since it would be less painful and could be done by the patient himself, with a better cost-benefit ratio. On the other hand, the s.c. route has been shown to produce more sustained and less fluctuating FSH concentrations as compared to those obtained by the i.m. route (Handelsman *et al.*, 1995).

Recently, a new FSH preparation, highly purified FSH (FSH-HP), has been made commercially available by Serono. Like HMG, FSH-HP is a gonadotrophin obtained from the urine of menopausal women but it is purified by specific anti-FSH monoclonal antibodies, giving a purer product that can be self-administered s.c. by the patient (Le Cotonnec *et al.*, 1993; Howles *et al.*, 1994).

The purpose of this study was to assess the efficacy and safety of combined treatment with highly purified FSH and HCG, both administered s.c., to stimulate testicular spermatogenesis and steroidogenesis in males suffering from HH with azoospermia or aspermia.

Materials and methods

Study design

The study was designed as a prospective, phase II–III, open, non-comparative, multicentre trial with patients serving as their own

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controls. The study was conducted between February 1992 and April 1996 in 14 Spanish centres. A total of 60 male patients with HH were planned to be included in the study, assigned to a single group treated with FSH-HP plus HCG.

The clinical trial was approved by the Ethics Committee of each participating centre as well as by the Directorate General of Pharmacy and Health Products of the Ministry of Health. The study was conducted in accordance with the Declaration of Helsinki and in compliance with good clinical practice. Each patient gave written informed consent before entering the study.

Patient selection

The study population consisted of males suffering from HH with azoospermia or aspermia, aged between 18 and 45 years, with low plasma testosterone concentrations (<200 ng/dl) which responded to HCG stimulation, and plasma LH/FSH concentrations below or at the lower normal limit; a washout period of at least 2 months for previous treatment with testosterone/HCG and 6 months for gonadotrophin releasing hormone (GnRH) or HMG was established. Exclusion criteria were: relevant systemic diseases or treatments which might influence the study results or drug pharmacokinetics; body mass index (BMI) >28.3; existence of other non-treated pituitary deficiencies; hyperresponse to the GnRH stimulation test; infection of genital tract; bilateral anorchia; mechanical abnormalities impairing sperm collection; hyperprolactinaemia; varicocele; cryptorchidism; intellectual deficiency or inability to comply with the study procedures.

Study drugs

FSH-HP (Metrodin HP®, Serono, Madrid, Spain) was supplied as lyophilized powder in ampoules, each containing 75 or 150 IU FSH (batch nos 0615/s, 17501042, 17504082, 17521123 and 17502035). HCG (Profasi HP®, Serono) was supplied as a lyophilized powder in ampoules, each containing 2500 IU HCG (batch nos P-5371, I-5 and J-2).

Treatment schedule

Before starting combined gonadotrophin treatment, written informed consent was obtained from each patient and compliance with elegibility criteria was verified; this included a positive testosterone response to HCG administration (2500 IU, twice a week) over 4 weeks. Then, all eligible patients self-administered s.c. injections of FSH-HP (150 IU, three times a week) and HCG (2500 IU, twice a week). The HCG dose was reduced in three patients due to abnormal testosterone responses or gynaecomastia. Combined treatment needed to be carried out for at least 6 months; at the end of this period, if no adequate spermatogenic response had been obtained, treatment was to be extended for at least 3 additional months.

End points

The primary efficacy end point was the spermatogenic response in terms of sperm concentration. The response was defined as complete if a sperm concentration of at least 1×10^6/ml was achieved during treatment, whereas it was considered as partial in the case of sperm appearance in the ejaculate with a concentration $<1 \times 10^6$/ml. Secondary end points for efficacy included total sperm count, sperm quality in terms of motility, morphology and viability, ejaculate volume, plasma testosterone concentrations, testicular size and secondary sexual characteristics.

Safety was assessed in terms of adverse events, physical examination (including injection site) and routine laboratory tests, including haematological parameters (red blood cell count, haemoglobin, haematocrit, leukocyte numbers and differential count and platelets), blood chemistry [glucose, creatinine, total proteins, bilirubin, serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), alkaline phosphatase, cholesterol and uric acid] and urinalysis (proteinuria, glucosuria, ketonuria, pH and density).

Evaluation methods

At screening, a medical history was obtained and a physical examination was performed. In addition to routine laboratory parameters, baseline hormonal concentrations of prolactin, thyroid stimulating hormone (TSH), thyroid hormones, and cortisol were determined. LH and FSH concentrations were measured basally and after GnRH stimulation, and plasma testosterone concentrations were estimated also basally and after HCG stimulation, as follows: after a basal plasma sample was collected, 2500 IU HCG were administered s.c. twice a week for 4 consecutive weeks. Three days after the last HCG injection, blood was obtained again, spun and the plasma used to measure post-stimulation testosterone concentrations. At all time-points at least two blood samples were collected with a ⩾20 min interval and were assayed either separately or as a pool. In the case of independent assay, testosterone concentration was defined as the mean of values obtained in each aliquot. Testosterone concentrations were measured in plasma obtained from heparin- or EDTA-treated blood. Measurements were performed using a commercially available radioimmunoassay method. Testosterone measurements were repeated after 1, 3, 6 or 9 months of treatment.

Spermiograms were performed under basal conditions (two spermiograms at least 7 days apart within 3 months before entering the study), and then monthly from the third month of treatment onwards. Seminal fluid was collected by masturbation after 3–5 days of abstinence using glass or plastic containers, avoiding the use of condoms. Sperm samples were immediately transported to the laboratory, avoiding delays of >1 h or exposure to extremes of temperature. Spermiograms were assessed according to the World Health Organization (WHO, 1992) criteria, using a haemocytometer to measure sperm concentration.

To assess testicular size, the short and long axis of each testis was measured with a caliper basally and at each subsequent visit. Testicular volume was calculated according to the following formula (Ley and Leonard, 1985):

$$V = 4/3\ \pi\ (a/2)^2\ b/2$$

where a represents the short testicular axis and b the long testicular axis (in cm). Volumes (V) are expressed in ml, as the average of both testicles.

Penis length was measured at the beginning of the study and at each subsequent visit using a caliper. Pubic hair was assessed initially and at each subsequent visit using Tanner's classification. At the end of the treatment period, testicular biopsy samples were taken in some of the patients who had not responded.

Adverse events and physical examination results were recorded at each visit. Routine laboratory tests were performed basally and periodically during treatment.

Statistical methods

The protocol aimed for the inclusion of at least 60 evaluable patients, since this sample size was considered to be representative of the studied population, taking into account the low incidence of HH. To assess treatment efficacy, the proportion of patients achieving a spermatogenic response and the corresponding 95% confidence interval (CI) were calculated. Basal values of sperm concentration and total sperm count were compared with maximum values obtained after treatment, using Wilcoxon's matched pairs signed-ranks test. Testosterone plasma concentrations at the different time-points were

Table I. Demographic and epidemiological characteristics ($n = 60$)

Age (years, mean ± SEM)	26.3 ± 0.85
BMI (kg/m^2, mean ± SEM)	24.0 ± 0.38
Age at diagnosis (years, mean ± SEM)	18.1 ± 0.8
Diagnosis (*n*, %)	
Kallmann's syndrome	16 (26.7)
idiopathic HH	19 (31.7)
multiple pituitary deficiency	25 (41.7)
Onset of hypogonadism (*n*, %)	
prepubertal	52 (86.7)
postpubertal	8 (13.3)
Previous treatment (*n*, %)	
testosterone	21 (35.0)
gonadotrophins	10 (16.7)
testosterone + gonadotrophins	26 (43.3)
none	3 (5.0)
Testicular volume (ml, mean ± SEM)	4.3 ± 0.5
Baseline spermiogram (*n*, %)	
azoospermia	26 (43.3)
aspermia	34 (56.7)

analysed by Friedman's test and the evolution of ejaculate volume, testicular volume and penis size were analysed using analysis of variance (ANOVA) for repeated measures. Concerning pubic hair, baseline status was compared with that reached at the end of treatment, using Wilcoxon's matched pairs signed-ranks test.

To assess the influence of several prognostic factors, the response to treatment was compared in different diagnostic groups, as well as in terms of previous treatments, testicular volume, and pre- or post-pubertal onset of hypogonadism. Correlation between different variables was analysed using the Pearson *r* coefficient.

To assess treatment safety, further to calculating the incidence of adverse events and physical examination or laboratory findings, the evolution of laboratory parameters from baseline throughout the treatment was assessed by ANOVA for repeated measures.

Statistical significance was defined as a P value <0.05.

Results

Study population

Treatment was initiated in 63 patients, but three of them were withdrawn from the study because of ineligibility, severe headache and personal reasons respectively, before any efficacy assessment could be performed. Thus, 60 patients could be assessed for efficacy while 63 patients were considered for the safety analysis.

Treatment compliance was correct in the vast majority of patients, with only three patients being considered as poor compliants.

Demographic and other baseline characteristics

Demographic, epidemiological and baseline characteristics of the 60 patients included in the efficacy analysis are summarized in Table I. The study sample consisted of relatively young patients, 35 showing isolated HH (Kallmann's syndrome or idiopathic HH) and 25 with hypopituitarism. The disease started before puberty in 52 patients. Most patients had been previously treated with testosterone, either as a single treatment or preceded by gonadotrophin administration, while three patients had never been treated.

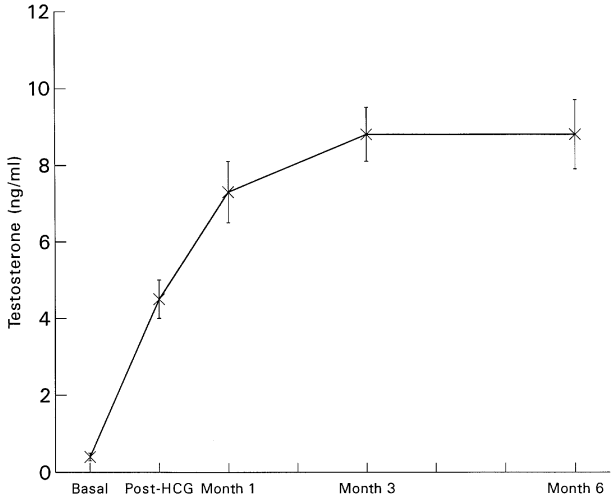

Figure 1. Plasma testosterone concentrations (mean ± SEM) at baseline, post-HCG and during the course of treatment.

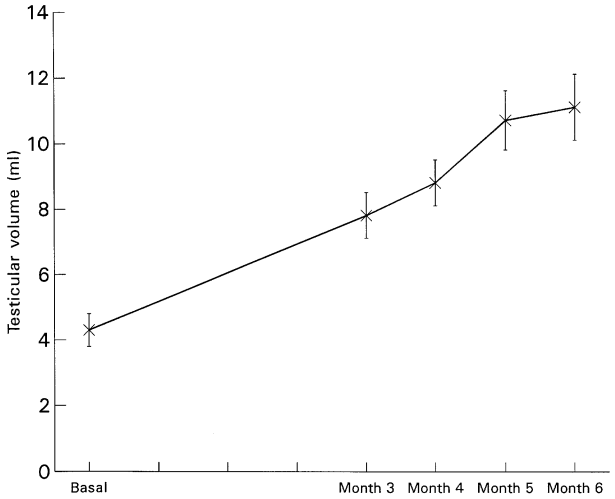

Figure 2. Testicular volume (mean ± SEM) before and during treatment.

Testosterone plasma concentrations

At the beginning of the study, all patients had testosterone concentrations far below the normal range (0.4 ± 0.1 ng/ml, mean ± SEM). As shown in Figure 1, testosterone plasma concentrations increased considerably in response to the HCG test and continued increasing during combined treatment with HCG and FSH-HP reaching a value of 8.8 ± 0.9 ng/ml (mean ± SEM) after 6 months of treatment ($P < 0.001$). All patients achieved testosterone concentrations within the normal range (>3 ng/ml), with the exception of one patient with a minimal testicular volume (0.4 ml), whose testicular biopsy after 9 months of treatment revealed interstitial fibrosis and absence of Leydig's cells in both testes.

Testicular volume

Figure 2 shows the evolution of testicular volume, which experienced a sustained and significant increase with the treatment from 4.3 ± 0.5 ml at baseline to 11.1 ± 1.0 after 6 months ($P < 0.001$). All but one patient experienced an increase during treatment and 14 achieved a normal testicular volume

Table II. Patients showing spermatogenic response to gonadotrophin treatment ($n = 60$)

	n	%	95% confidence interval
Complete response ($\geq 1 \times 10^6$/ml)	39	65.0	51.5–76.5
Partial response (>0 and $<1 \times 10^6$/ml)	9	15.0	7.5–27.1
Overall response (positive sperm count)	48	80.0	67.3–88.8
No response	12	20.0	11.2–32.7

for a healthy adult (>16 ml). The testicular volume at 6 months of treatment was significantly correlated with that shown at baseline ($r = 0.5$; $P < 0.01$).

Spermatogenic response

At the beginning of the study, only 26 patients (43.3%) were able to ejaculate and all of them showed azoospermia, while the rest of patients were aspermic. After 6 months of treatment, 28 patients (46.7%) had achieved a sperm concentration $>1 \times 10^6$/ml and all of them finished the study at this point except four patients who were willing to continue therapy.

The remaining 32 patients who had not achieved an adequate response were asked to extend treatment for at least 3 additional months. Three of these patients refused to continue therapy for personal reasons and another one was withdrawn from the study because of an adverse event, so 28 out of 32 patients with no adequate response continued treatment beyond 6 months.

Table II shows the proportion of patients who achieved a spermatogenic response during the study period. At the end of treatment, an adequate response was observed in 39 (65%) patients while another nine patients showed a positive sperm count with a sperm concentration $<1 \times 10^6$/ml. Thus, the overall response rate was 48/60 (80.0%) (95% CI: 67.3–88.8%). Testicular specimens were obtained in those non-responders who accepted biopsies; their examination generally revealed testicular fibrosis and/or spermatogenesis arrested at early stages.

A survival analysis of time required to induce spermatogenesis is shown in Figure 3. The median time to achieve a positive sperm count was 5 months (95% CI: 3.31–6.69 months).

The evolution of sperm concentration throughout the study period is shown in Figure 4. All patients who were able to ejaculate basally ($n = 26$) showed azoospermia. Sperm concentration increased progressively over the treatment period achieving an average maximum value of $24.5 \pm 8.1 \times 10^6$/ml (mean ± SEM), significantly higher ($P < 0.001$) than the pre-treatment value. In all, 14 patients reached a sperm concentration within the normal range ($>20 \times 10^6$/ml, according to WHO criteria). Total sperm count showed a similar pattern, increasing from a 0 value at baseline up to a maximum of $59.8 \pm 19.7 \times 10^6$ spermatozoa per ejaculate (mean ± SEM) during treatment ($P < 0.001$).

Sperm motility, morphology and viability could not be assessed at baseline since all the patients capable of ejaculating presented with azoospermia. After 3 months of treatment,

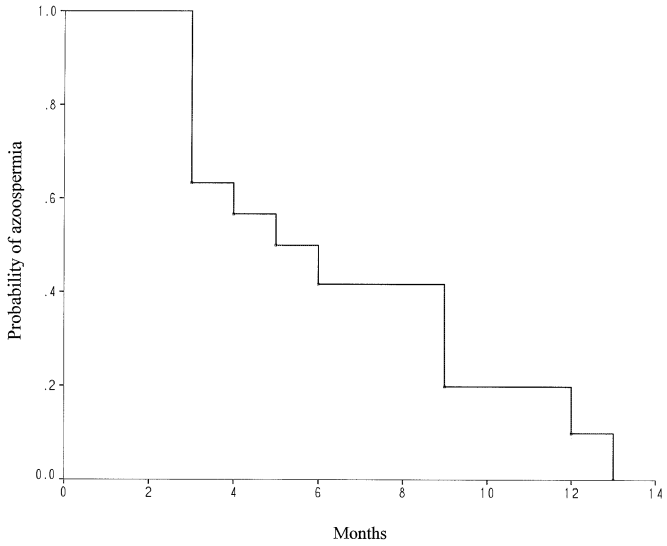

Figure 3. Survival analysis of time to induce spermatogenesis.

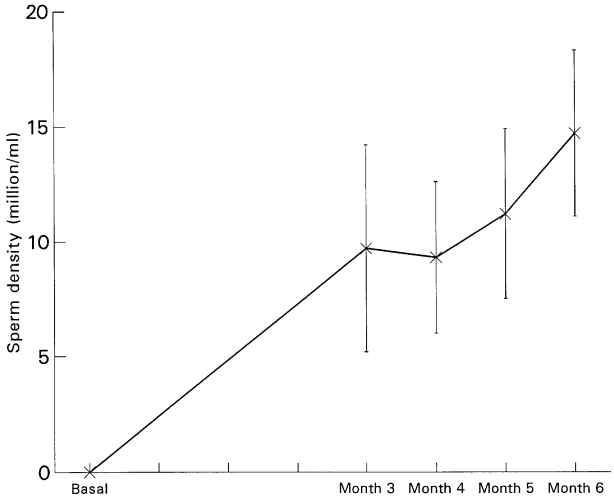

Figure 4. Evolution of sperm concentration (mean ± SEM) throughout the treatment period.

motility was observed in $44.1 \pm 4.0\%$ (mean ± SEM) of spermatozoa, with a normal morphology in $56.4 \pm 8.5\%$ and viability in $64.3 \pm 4.9\%$ of spermatozoa. These values did not show significant changes at subsequent assessments throughout the treatment period.

Ejaculate volume

Prior to the beginning of treatment, the ejaculate volume in those patients who produced sperm samples was 1.2 ± 0.2 ml (mean ± SEM). Ejaculate volume was promptly and significantly ($P < 0.001$) increased by gonadotrophin treatment so that a normal volume was achieved, on average, at 3 months (2.4 ± 0.2 ml, mean ± SEM) and was maintained over the treatment period.

Out of 34 patients who were not able to ejaculate at the beginning, 30 started to do so during treatment and reached normal volumes.

Secondary sexual characteristics

Penis length increased significantly ($P < 0.001$) over the treatment period from 6.0 ± 0.3 cm (mean ± SEM) at baseline to 8.1 ± 0.3 cm at 6 months. Regarding pubic hair, prior to starting treatment 20% of patients were prepubertal (Tanner's stage I–II). However, after 5 months of treatment all patients had reached Tanner's stage III–V ($P < 0.01$).

Pregnancies

Most patients included in the study were not interested in immediate procreation. Only four patients manifested their desire to father children and the partner of one of them became pregnant during the study and gave birth to a normal child. Pregnancy was achieved in the fourth month of treatment when the sperm concentration was 4.9×10^6/ml.

Prognostic factors

No significant differences were observed in the spermatogenic response between the different diagnostic groups. The rate of complete responses in patients with isolated HH (63%) was quite similar to that observed in patients with multiple pituitary deficiencies (68%). Treatment efficacy was significantly related to the onset of hypogonadism. Thus, in patients diagnosed of hypogonadism before puberty the response rate at the end of treatment was of 59.6%, whereas 100% of the eight patients with hypogonadism of postpubertal onset showed an adequate response at 6 months ($P < 0.01$). Likewise, maximum sperm concentration in patients with postpubertal onset hypogonadism ($37.9 \pm 14.5 \times 10^6$/ml) was significantly higher ($P = 0.04$) than that observed in the rest of patients ($22.3 \pm 8.5 \times 10^6$/ml, mean ± SEM).

The spermatogenic response was not significantly related to previous treatments received by patients, although there was a trend to a better response in patients treated with gonadotrophins. Thus, nine out of 10 (90%) patients who were receiving gonadotrophins before entering the study achieved spermatogenesis as compared to 78% of the rest of patients ($P = 0.3$). Likewise, the overall response rate in patients who had received FSH preparations was slightly higher than in the rest of patients (84.2 versus 77.5%) but the difference was not significant.

Neither the response rate nor maximum sperm concentration showed a significant association with basal or post-treatment testicular size. Finally, the response rate in those patients who were able to ejaculate at the beginning of the study (overall response of 88.5%, 95% CI: 68.7–96.9%) was not significantly different from that observed in the subgroup of patients that could not ejaculate at baseline (overall response of 73.5%, 95% CI: 55.3–86.5%).

Safety

Safety was assessed in a total of 63 patients who received at least one dose of FSH-HP. Only 11 patients (17.5%) reported some adverse event which, in most cases, was not serious and not considered as related to treatment. These included acute hepatitis B, trauma, chest pain, dizziness, traumatic orchitis and biochemical abnormalities (increase in bilirubin, cholesterol or uric acid). Six patients (9.5%) suffered from adverse events which could be related to the combined treatment with FSH-HP + HCG. One of them presented with intense headache leading him to withdraw from the study. Three patients showed mild or moderate bilateral gynaecomastia, which improved in one case after HCG dose reduction. One patient suffered from acne, probably related to HCG administration. Only two serious adverse events were observed during treatment, both occurring in the same patient; the first event was a surgical intervention due to coxa vara, not related to the study drugs; then, after 6 months of treatment, the patient presented with an episode of benign intracranial hypertension, considered as possibly related to treatment, which caused the patient's withdrawal from the study and improved after corticosteroid administration.

Most patients showed some abnormalities in haematological or biochemical parameters, either before or during treatment, which in general were not clinically relevant. Nine patients had a low basal red blood cell count, frequently accompanied by a decrease in haemoglobin and/or haematocrit values, which generally improved during treatment. ANOVA showed a significant increase in mean red blood cell count (from 4.64 ± 0.4 at pre-treatment to $5.0 \pm 0.4 \times 10^6$/μl at 6 months), haemoglobin (from 13.8 ± 1.1 to 15.0 ± 1.3 g/dl) and haematocrit (from 40.5 ± 3.2 to 44.3 ± 3.4%) over the treatment period. There was also a significant increase in creatinine, uric acid and alkaline phosphatase, whereas mean concentrations of cholesterol, SGOT and SGPT were significantly decreased by treatment. Physical examination, heart rate and blood pressure were always normal. At all visits the injection site was carefully examined with no local reactions observed in any patient.

Discussion

To the best of our knowledge, the present study represents the largest therapeutic trial in men with HH ever reported in the literature.

Regarding the testicular steroidogenic response, testosterone reached normal values in nearly all patients, which is consistent with the data published by other authors (Finkel *et al.*, 1985; Ley and Leonard, 1985; Saal *et al.*, 1991; Mastrogiacomo *et al.*, 1991). These figures are higher than those obtained by Okuyama *et al.* (1986) in patients with HH whose mean testosterone concentrations were still below the normal range after 6 months of treatment with HCG and HMG. Likewise, the response rate of the present study is higher than that obtained by Kirk *et al.* (1994) in patients with HH treated with slightly lower HCG doses.

The testicular volume experienced a dramatic, almost 3-fold increase during treatment, although most patients did not reach normal values, which agrees with the results obtained by others (Ley and Leonard, 1985; Liu *et al.*, 1988; Saal *et al.*, 1991; Kliesch *et al.*, 1994) in patients treated with HMG + HCG.

The spermatogenic response observed with the new preparation of highly purified FSH was comparable or even better than that reported in patients treated with HMG + HCG (Saal *et al.*, 1991; Schopohl *et al.*, 1991; Kirk *et al.*, 1994).

Although most patients did not reach a normal sperm concentration ($\geqslant 20 \times 10^6$/ml), normal values are currently

thought not to be absolutely necessary to achieve fertility (Van Zyl *et al.*, 1975; Zukerman *et al.*, 1977; Sheriff, 1983; Ley and Leonard, 1985; Sokol and Sparkes, 1987; Kliesch *et al.*, 1994). This is particularly true in patients with HH who, after treatment with gonadotrophins, usually become fertile with sperm concentrations far below 20×10^6/ml (Burris *et al.*, 1988).

On the other hand, and taking into account the spermatogenic process, treatment duration in the present study was too short to normalize sperm concentration, since most of the enrolled patients were not seeking fertility at that time and the aim of the study was just to assess the ability of the treatment to induce spermatogenesis. A longer treatment period would certainly have been required to normalize sperm concentrations, as shown by Okuyama *et al.* (1986) who only achieved it in 10 out of 18 patients with HH after 24–48 months of HMG + HCG treatment.

The time required to obtain a positive sperm count was shorter in the present clinical trial (median of 5 months) than in other studies. Kliesch *et al.* (1994) described a mean time of 8.7 ± 4.8 months to induce spermatogenesis in patients with isolated HH and of 6.7 ± 4.8 months in those with hypopituitarism.

The response rate was quite similar in patients with hypopituitarism and in those with isolated HH. Literature reports are not consistent on this issue, since some authors found a better response in patients with multiple pituitary deficiencies (Okuyama *et al.*, 1986; Kliesch *et al.*, 1994) while others (Ley and Leonard, 1985) observed a poorer sperm response in these patients.

The spermatogenic response was not significantly dependent on the previous treatments, which is consistent with data from others (Ley and Leonard, 1985; Kliesch *et al.*, 1994). The response rate was slightly higher in patients who were receiving gonadotrophins before their inclusion in the study and particularly in those who had received FSH preparations, but the difference was not significant, possibly due to the relatively small number of patients in this situation.

The response was better in patients with postpubertal HH than in those with a prepubertal onset, as shown by other authors (Finkel *et al.*, 1985; Ley and Leonard, 1985; Mastrogiacomo *et al.*, 1991).

Concerning sperm quality, the rate of motile, normal and viable forms observed during treatment with FSH-HP was similar to that observed with HMG (Kliesch *et al.*, 1994).

As far as safety is concerned, treatment was well tolerated with a low incidence of adverse events, most of which were not related to the study drug. In the present study only three cases of gynaecomastia were observed; this incidence is lower than that described by others (Schopohl *et al.*, 1991; Kliesch *et al.*, 1994; Kirk *et al.*, 1994) in patients treated with HMG + HCG and corresponds to the physiological gynaecomastia observed during puberty.

Treatment with gonadotrophins caused a significant increase in red blood cell count, haemoglobin and haematocrit, which is consistent with an improvement of the discrete anaemia presented by various patients prior to treatment. This phenomenon could be due to the stimulating effect of testosterone on erythropoiesis (Wilson, 1991). The increase in uric acid and creatinine could be related to anabolic effects of treatment. Alkaline phosphatase increase was probably associated with body growth.

Local tolerance to s.c. injections was excellent with no reactions at the injection site.

Treatment compliance was good with a very low rate of drop-outs, in spite of repeated injections administered for months. This confirms the great advantage of s.c. self-administration, allowed by the high purity of FSH.

In conclusion, combined treatment with FSH-HP + HCG is effective and safe to stimulate spermatogenesis, steroidogenesis and testicular growth in patients suffering from HH.

Acknowledgements

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Everything That's Wrong With Your PCT by Eric M. Potratz

In the world of steroid users, it has become mandatory to follow post cycle therapy (PCT) upon cessation of steroid use. Many great PCT protocols have been outlined over the years, and many individuals have had great success with following such protocols. Nevertheless, what works can always work better. This is especially the case for those that have had a lack of success following popular advice. In this article I will address the major problems with popular PCT protocols and clarify exactly how we should use the items at our disposal for optimum recovery from AAS. Three main topics will be covered in this article –

hCG on cycle -- I will show you the best way to use HCG, which will protect your "testicular real-estate", and prime your HPTA for the fastest and most complete recovery possible.
SERMs. -- Drugs such as Clomid and Nolvadex are some of the most toxic drugs in a steroid-users cabinet. I will present the evidence of this toxicity and provide alternatives.
Peptides for PCT -- Peptides such as Growth Hormone and IGF-1 have much more of a role in PCT than most people realize. Besides preserving muscle gains, these hormones can actually help restore testicular function after a cycle.

HCG unraveled

Human Chorionic Gonadotropin (hCG) is a peptide hormone that is used in place of LH to stimulate hormone production from the gonads.1 LH is the primary signal sent from the pituitary to the testes, which stimulates the leydig cells within the testes to produce testosterone. When steroids are administered, LH levels rapidly decline. The absence of an LH signal from the pituitary causes the rapid onset of testicular degeneration. The testicular degeneration begins with a reduction of leydig cell volume, and is then followed by rapid reductions in intra-testicular testosterone (ITT), peroxisomes, and Insulin-like factor 3 (INSL3) – All important bio-markers and factors for proper testicular function and testosterone production.2-6,19 However, this degeneration can be prevented by a small maintenance dose of hCG ran throughout the cycle. Unfortunately, most steroid users have been engrained to believe that hCG should be used after a cycle. Though, we will learn that a faster and more complete recovery is possible if hCG is ran during a cycle.

Firstly, we must understand the clinical history of hCG to understand the most efficient way to use it. Many popular "steroid profiles" advocate an hCG dose of 2500-5000iu once or twice a week. These were the kind of dosages used in the historical hCG studies for hypogonadal men who had reduced testicular sensitivity due to prolonged LH deficiency.85,86 That is, testes desensitize when not presented with a sufficient LH signal. In men with normal LH levels and testicular sensitivity, the maximum increase of testosterone is seen from a dose of only ~250iu, with minimal increases obtained from 500iu or even 5000iu.2,11 (It appears the testes maximum secretion of testosterone is about 140% above base line.12-18) So, if you have allowed your testes to desensitize over the length of a typical steroid cycle, (8-16 weeks) then you would require a higher dose to elicit a response in an attempt to restore normal testicular size and function – but there is cost to this, and a high probability that you won't regain full testicular function.

To get an idea of how quickly testicular degeneration occurs from your average multi-AAS cycle, consider this: LH levels are rapidly decreased by the 2nd day of steroid administration.2,9,10 By shutting down the LH signal and allowing the testis to be non-functional over a 12-16 week period, leydig cell volume decreases 90%, ITT decreases 94%, INSL3 decreases 95%, while the capacity to secrete testosterone decreases as much as 98%.2-6 It should be mentioned that visually analyzing testes size is a poor method of judging your actual testicular function, since testicular size is not directly related to the ability to secrete testosterone.4 This is because the leydig cells, which are the primary sites of testosterone secretion, only make up about 10% of the total testicular volume. Therefore, testicular size may appear normal on a cycle, but the testes ability to secrete testosterone upon LH or hCG stimulation can actually be significantly diminished.3-5

The decreased testosterone secretion capacity was well demonstrated in a study on power athletes who used steroids for 16 weeks, and were then administered 4500iu hCG post cycle. It was found that the steroid users were about 20 times less responsive to hCG, when compared to normal men who did not use steroids.8 In other words, their testosterone secretion capacity was dramatically reduced because they did not receive an LH signal for 16 weeks. The testes essentially became desensitized and crippled. Case studies with steroid using patients show that aggressive long-term treatment with hCG at dosages as high as 10,000iu E3D for 12 weeks were unable to return full testicular size.7 Other studies with men using low dose steroid implants for 6 weeks showed unsuccessful return of Insulin-like factor-3 (INSL3) concentration in the testes upon 5000iu/wk of HCG treatment for 12 weeks.6

These studies show that postponing hCG usage until the end of a cycle, increases your need for a higher

dose of hCG, and decreases your odds of a full recovery. As a consequence to using a higher dose of hCG, estrogen will be increased disproportionately, which then causes further HPTA suppression while increasing the risk of gyno.11 For example, high doses of hCG are known to raise estradiol 165%, while only raising testosterone 140%.11 Higher doses of hCG are also known to reduce LH receptor concentration and degrade the enzymes responsible for testosterone synthesis within the testes12,13,19 (the last thing someone wants during recovery). While these negative effects of hCG can be partly mitigated by the use of a drug such as tamoxifen, it will create further problems associated with using a toxic SERM. (covered in the next section)

In light of the above evidence, it becomes obvious that we must take preventative measures to avoid this testicular degeneration. Besides, with hCG being so readily available, and such a painless shot, it makes you wonder why anyone wouldn't use it on cycle. Based on studies with normal men using steroids, ~100iu HCG administered everyday was enough to preserve full testicular function and ITT levels, without causing desensitization typically associated with higher doses of hCG.2 It is important that low-dose hCG is started before testicular degeneration occurs, which appears to rapidly manifest within the first 2-3 weeks of steroid use.

Recap – For optimal preservation of testicular function during cycle, use 100iu hCG ED starting 3 days after your first AAS dose. Drop the hCG a week before the AAS clear the system. For example, you would drop hCG a week after your last Testosterone Enanthate shot. Or, if you are ending the cycle with orals, you would drop the hCG a week before your last oral dose. This will allow for a sudden and even drop in hormone levels, while initiating LH and FSH production from the pituitary, making for a seamless recovery.

A more convenient alternative to the above recommendation would be a weekly shot of 500iu hCG, throughout the entire cycle. Beyond this dose, one could calculate a rough estimate for their required hCG dosage by multiplying 40iu x days of LH absence. (40iu x 60 days = 2400iu HCG dose)

As an alternative to the on cycle hCG protocol, you could follow a plan based on modulation of the gonadotropin pulse generator. (seen here)

Note: If following any of these protocols, hCG should NOT be used after the cycle.

Clomid & Nolva; A closer look

The use of Clomid and Nolvadex, as Selective Estrogen Receptor Modulators (SERMs), has gradually become well established in the steroid using community. The popular push of these drugs has almost made them mandatory. They have essentially become hormonal vitamins – vitamins that can do no wrong and provide seemingly endless benefits of testosterone support, bloat reduction, gynecomastia prevention and cholesterol health. It seems that we are all well educated about the benefits of Clomid and Nolvadex, so in this segment, I will present the risks and consequences from the short and long term use of Clomid and Nolvadex.

Upon examination of the research available for Clomid (clomiphene) and Nolvadex (tamoxifen) we find that the research is quite extensive, and contradicting.21 We see many early studies with tamoxifen done on breast cancer patients, which show an acceptable "safety profile", with an apparent lack of adverse effects.22 On the other hand, many of the early in vivo animal studies showed severely toxic effects, with the development of cancer in the liver, uterus, or testes upon tamoxifen administration.30-34,41 However, this evidence was largely disregarded by ex vivo (test tube) research on human cell-lines which appeared to show a lack of toxic effects.21

For example, tamoxifen was generally accepted as being non-toxic to human liver upon the conclusion that tamoxifen did not cause noticeable DNA adducts (damage) during short-term ex vivo studies with human liver cells.35,36 This was in contrast to the in vivo animal studies showing dramatic carcinogenic effects on the liver.30-34,41 As scientists learned that the toxic effects from tamoxifen are from the metabolism and buildup of the a-hydroxytamoxifen, 4-hydroxytamoxifen and N-desmethyltamoxifen metabolites. It became apparent that ex vivo research was largely flawed due to low-rate metabolism.21 The carcinogenic effects of tamoxifen proved to be even more unusual and elusive, when it was hypothesized that tamoxifen had both genomic and non-genomic toxicity, which affecting different animals, in different organs.21 This created an obvious clinical challenge for measuring genotoxicity in a test tube. Eventually, it was established that tamoxifen was a bona-fide carcinogen in all species, at least in one way or another.21,37-39 Recent human studies have shown tamoxifen treated women to have 3x the risk of developing fatty liver disease, which appeared as soon as 3 months into therapy at only 20mg/day.24-26 In some cases, the disease lasted up to 3 years, despite cessation from tamoxifen therapy. Five and ten year follow-ups with

patients on long term tamoxifen therapy showed cases of deadly hepatocellular carcinoma.27-29 In a 2000 case study involving tamoxifen induced liver disease, D.F Moffat et al made a profound statement –

"In addition, hepatocellular carcinoma in tamoxifen treated patients may be under-reported since there may be reluctance to biopsy liver tumours which are assumed to be secondary carcinoma of the breast."

In other words, it appears that the liver carcinoma from a large number of breast cancer patients on tamoxifen therapy has been misdiagnosed as a metastasis infection from the breast cancer itself.28 Upon closer examination it was found that the cancerous lesions in the livers of the long-term tamoxifen therapy case studies were identical to those seen in the early animal studies showing tamoxifen to be a potent hepatotoxin.28-34 Although the effects took much longer to manifest, it became obvious that tamoxifen was toxic to the human liver.

Another well known risk of tamoxifen therapy is the increased risk of developing endometrial cancer (uterine cancer).23,42 This is due to tamoxifen actually acting as an estrogen agonist in the uterus, presumable from the 4-hydroxytamoxifen metabolite.33,40 This estrogenic metabolite triggers abnormal growth of the uterus and the formation of cancer causing DNA adducts.33 As male bodybuilders we assume this presents no risk. On the contrary, the implications are quite scary when we realize the male equivalent to the uterus is the prostate -- differentiating from the same embryonic cell line and sharing the same oncogene, Bcl-2, and high concentration of the estrogen receptor. It is likely that tamoxifen has the same estrogenic action, and DNA damaging effects within the prostate.60-62 It is no wonder that tamoxifen failed as a treatment for prostate carcinoma.43

Aside from restoring testosterone levels post cycle, tamoxifen is often used to combat gyno during cycle when "flare ups" occur. While tamoxifen may provide immediate inhibition of growth, and serve as valuable tool, it also has the ability to up-regulate the progesterone receptor.54-56 This is a true contradiction, which dramatically increases your chances of bringing upon gyno in future cycles when utilizing Nandrolone (Deca) or Trenbolone, both of which act upon the progesterone receptor. It is interesting to speculate: is tamoxifen use directly related to the increased gyno occurrences seen with modern day steroid users?

When we bring our attention to Clomid, we find less research is available on long term human toxicity, probably because of the relatively short term (3-4 week) clinical application for ovarian stimulation,59 although long term follow ups with patients who received Clomid for ovulation induction have shown an increased risk of developing uterine cancer.74 This is to be expected, since many of the same carcinogenic tendencies found with tamoxifen are the same effects seen with clomiphene.44,45,57,58 Upon analysis of anecdotal reports from Clomid and nolva users, we see the typical short term side effects of low libido, erectile dysfunction, and emotional instability – despite many men showing normalized testosterone and estrogen levels during the use of these SERM's. Research on male breast cancer patients also shows frequent reports of low libido, thrombosis (arterial blockage), and hot flashes with tamoxifen use.47 Another common side effect associated with both SERMs, but more common with Clomid, is the loss of visual accuracy and development of visual "tracers", due to the ocular toxicity.46

As the medical community became more aware of the side-effects associated with clomiphene and tamoxifen treatment, newer and safer SERMs, such as toremifene and raloxifene hit the developmental fast track. Toremifene appears to be less liver toxic, but it is an analog of tamoxifen, so it also carries many of the related genotoxic effects.48,49 Raloxifene appears to be even safer by being the least liver toxic, and not having any potential issue with the uterus or prostate.50-52 Unfortunately, raloxifene has been associated with a higher incidence of thromboembolism52 (arterial blockage), and also has very low oral absorption, making it an expensive alternative at a typical 120mg/day dose.53 Still, raloxifene could presumably be equally effective as Clomid or Nolvadex at restoring HPTA function, while imparting less side effects.53 Newer SERMs are already being evaluated such as bazedoxifene, arzoxifene, and lasofoxifene, in hopes of reducing risk even further.

Another SERM that may be useful for post cycle therapy is resveratrol.87,88 Resveratrol is a natural polyphenol extracted from grape skin, that has recently been under heavy research for its cancer fighting effects in the breast, prostate and liver.63-69 Contrary to Nolva or Clomid, resveratrol appears to actually have beneficial effects on the liver,70 as well as having multiple benefits on cardiovascular health by limiting LDL oxidation and improving endothelial function.71-73 Improved blood vessel function may be a mechanism by which resveratrol improves erectile function in many men. Research also suggests that resveratrol may actually extend life, by reducing oxidative stress on organs such as the heart,77 and preventing the metabolic syndrome by fighting insulin resistence.79,80 It's becoming well known that insulin resistance is a leading cause of low testosterone.82 More specifically, improving insulin sensitivity

will increase your leydig cell sensitivity, and therefore increase the testes response to LH.81

It should be pointed out that resveratrol may not be the best choice to combating emergency gyno, due to its lower binding affinity to the human ER of about 90x less than tamoxifen, and about 30x less than clomiphene.75,76 However, considering that resveratrol is a pure estrogen antagonist at the pituitary,89 while Clomid has mixed agonist/antagonistic effects,90-94 resveratrol could be a suitable substitute for PCT. Aside from acting as a SERM, resveratrol can also help control estrogen by actually limiting aromatase enzyme production.82 Based on the research, it appears that at least 100mg/day would needed to increase LH, FSH and testosterone production.84

Admittedly, no steroid users are dropping dead from a 4 week protocol of Nolva or Clomid, and many will say "the consequences far outweigh the benefits" -- but why deal with the potential consequences when alternatives are available?

Peptides for testicular recovery

It's a common practice these days for experienced bodybuilders to implement some dosage of IGF-1 either during or after a cycle to "pick up" a lagging body part, or to preserve gains in muscle. Growth Hormone (GH) is also a versatile drugd for cutting or bulking, with increasing popularity as it becomes more affordable. The value of IGF-1 and GH becomes so much more significant when we realize there integral role in testicular function. In fact, it seems that these hormones are more effective at building testes, than muscles.

Research has shown GH to be vitally important in testicular function, 95-97 but it is generally accepted that the beneficial effects are directly mediated by hGH's conversion to IGF-1.98 As many of you know, IGF-1 is created in the liver by GH, upon interacting with insulin. So, we will be focusing on the usage and benefits of IGF-1, rather than GH, as it seems more cost effective and directly related to our purpose of optimizing recovery.

In short, IGF-1 increases steroidogenic acute regulatory protein (sTAR),98 and cholesterol side chain cleaving enzyme (CYP 11A)99. These are both rate-limiting steps and are critical factors for converting cholesterol into hormones, such as testosterone. IGF-1 also has the ability to increase the concentration of steroidogenic enzymes in the testes, such as 3b HSD.100 IGF-1 can also increase the testes sensitivity to LH and hCG by increasing the number of LH receptors.99-102

These positive effects on testicular function make IGF-1 an ideal drug for PCT. A dose of IGF-1 Lr3 at 80mcg/day, split two times per day, would likely be the most cost effective dose.

In conclusion, we have learned that utilizing hCG during a steroid cycle will significantly prevent testicular degeneration. This helps create a seamless transition from "on cycle" to "off cycle". Then, by avoiding the deleterious SERMs such Clomid and Nolvadex and opting for safer alternatives, you can seemingly avoid any sort of post cycle crash, while maintaining a strong libido and uncompromised emotional health.

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HPGA Normalization Protocol After Androgen Treatment

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Program for Wellness Restoration, PoWeR

Objective

To develop an approach to cycle androgens that would result in significant changes in body composition and accelerate the normalization of the hypothalamic pituitary gonadal axis (HPGA) after cessation of androgens.

Methods

An uncontrolled study of 19 HIV-negative eugonadal men, ages 23 – 57 years, administered testosterone cypionate and nandrolone decanoate for 12 weeks, and then were treated simultaneously with a combined regimen of human chorionic gonadotropin (hCG) (2500 IU/QODx16d), clomiphene citrate (50 mg PO BID x 30d) and tamoxifen (20 mg PO QD x 45d), to restore the HPGA.

Results

Mean FFM by DEXA increased from 64.1 to 69.8 kg ($p<.001$); percent body fat decreased from 23.6 to 20.9 ($p<.01$); strength increased significantly from 357.4 lb to 406.4 lb ($p=.02$). No significant changes in serum chemistries and liver function tests were found. HDL-C decreased from a mean value of 44.3 to 38.0 ($p=.02$). Mean values for luteinizing hormone (LH) and total testosterone (T) were 4.5 and 460, respectively prior to androgen treatment. At the conclusion of the 12-week treatment with androgens the mean LH <0.7 ($p<.001$) and total testosterone was 1568 ($p<.001$). The mean values after treatment with the combined regimen were LH=6.2 and testosterone=458.

Discussion

The use of androgens has been reported to improve lean body mass, strength, sexual function, and mood accompanied by side effects caused by continuous uninterrupted use of these compounds (polycythemia, testicular atrophy, hypertension, liver dysfunction [oral androgens] and alopecia.) Androgen-induced HPGA suppression causes a severe hypogonadal state in most patients that often require an extensive period of considerable duration for normalization. This prevents most if not all individuals from cycling off these medications due to the adverse impact of this state on their previously gained LBM and quality of life. The protocol of hCG-clomiphene-tamoxifen was successful in restoring the HPGA within 45 days after androgen cessation. Further controlled studies are needed to determine if these results can be duplicated in HIV-positive subjects.

Patient General Characteristics

	Mean	SD	Range
Age (yrs)	36.4	9.6	23-57
Height (cm)	179.3	10.3	152.4-198.1
Weight (kg)	87.7	9.8	71.7-106.6
BMI (kg/m2)	27.5	4.7	22.3-42.8

Mean Hormone Levels During Program

	Initial		Day 30		Day 90		Final		Change		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
T	459.5	224.4	1568.0*	551.6	1262.9*	551.6	457.9	206.7	-1.6	17.7	NS
LH	4.5	1.9	<0.7*	-	<0.7*	-	6.2	5.5	1.7	3.6	NS

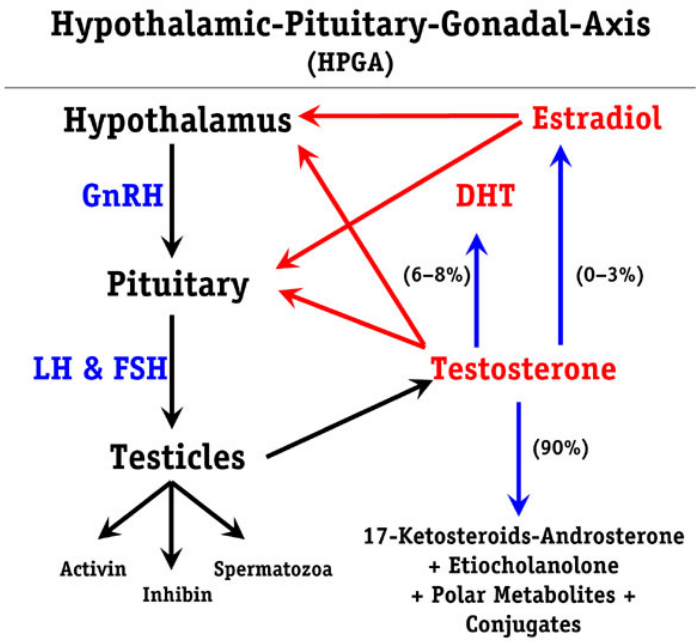
* P<.001 to initial value.

Serum Chemistry

	Initial		Final		Change		P
	Mean	SD	Mean	SD	Mean	SD	
BUN	20.1	5.5	20.7	6.2	0.6	0.7	NS
ALKP	75.8	15.4	65.6	20.1	10.2	4.8	.005
ALT	37.1	23.9	42.3	35.9	5.2	12.0	NS
AST	32.8	14.4	38.9	25.7	6.1	11.4	NS
Chol,Total	191.2	45.5	185.3	39.5	5.9	6.0	NS
HDL-C	44.3	14.1	38.0	11.2	6.3	2.9	.02
LDL-C	114.7	38.1	112.0	39.1	1.8	1.1	NS
TG	165.8	119.6	169.5	98.9	3.6	20.7	NS

Mean Body Composition & Strength

	Initial		Final		Change		P
	Mean	SD	Mean	SD	Mean	SD	
Fat (%)	23.6	7.2	20.9	4.8	2.8	2.4	.02
Weight (kg)	87.7	9.8	91.5	9.2	3.8	0.6	.001
Fat Free Mass (kg)	64.1	7.3	69.8	7.7	5.7	0.3	.001
Fat (kg)	23.4	7.0	21.6	5.0	1.9	1.9	NS
Σ Strength Tests (3)	357.4	125.6	406.4	151.4	49.0	25.9	.05



Androgens

(depoTestosterone; delaTestryl; Nandrolone Decanoate; Oxymetholone (Anadrol-50); Oxandrolone; Stanazolol (Winstrol).

Negative Effects:

- **Decreased GnRH; LH & FSH; Endogenous Testosterone; Testicular Size; & Spermatozoa.**
- **Increase Estradiol & DHT with depot and delaT.**
- **Androgen Induced Hypogonadism (AIH) of Unknown Duration & Severity to be Clinically Significant & Problematic to Preclude Androgen Cessation & Avoidance or Elimination of Negative Side-Effects of Androgens**

Medline Literature Search	
HPGA Normalization Studies For Androgens (Type, Dose, & Duration).	0
Medical Protocols/Interventions For HPGA Normalization.	0
Androgen Cycles Effecting Positive Body Composition Changes Combined w/ HPGA Normalization.	0
Medical Interventions To Eliminate/Minimize Adverse Side-Effects During Androgen Cycles.	0
Prevalence of Androgen Induced Hypogonadism Rx'ed w/ Testosterone …	

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Effect of Raising Endogenous Testosterone Levels in Impotent Men with Secondary Hypogonadism: Double Blind Placebo-Controlled Trial with Clomiphene Citrate

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ABSTRACT

Secondary hypogonadism is not an infrequent abnormality in older patients presenting with the primary complaint of erectile dysfunction. Because of the role of testosterone in mediating sexual desire and erectile function in men, these patients are usually treated with exogenous testosterone, which, while elevating the circulating androgens, suppresses gonadotropins from the hypothalamic-pituitary axis. The response of this form of therapy, although extolled in the lay literature, has usually not been effective in restoring or even improving sexual function. This failure of response could be the result of suppression of gonadotropins or the lack of a cause and effect relationship between sexual function and circulating androgens in this group of patients. Further, because exogenous testosterone can potentially increase the risk of prostate disease, it is important to be sure of the benefit sought, *i.e.* an increase in sexual function.

In an attempt to answer this question, we measured the hormone levels and studied the sexual function in 17 patients with erectile dysfunction who were found to have secondary hypogonadism. This double blind, placebo-controlled, cross-over study consisted of treatment with clomiphene citrate and a placebo for 2 months each.

Similar to our previous observations, LH, FSH, and total and free testosterone levels showed a significant elevation in response to clomiphene citrate over the response to placebo. However, sexual function, as monitored by questionnaires and nocturnal penile tumescence and rigidity testing, did not improve except for some limited parameters in younger and healthier men.

The results confirmed that there can be a functional secondary hypogonadism in men on an out-patient basis, but correction of the hormonal status does not universally reverse the associated erectile dysfunction to normal, thus requiring closer scrutiny of claims of cause and effect relationships between hypogonadism and erectile dysfunction. (*J Clin Endocrinol Metab* **80:** 3546–3552, 1995)

FOR MANY YEARS, investigators have tried to define a male counterpart to the female menopause (1, 2), and after much debate for the past 2 decades, it has become clear that levels of testosterone decrease slowly in men after the age of 40 yr (3). Further, the fall is greater in the free fraction of testosterone because of a rise in sex hormone-binding globulin (4).

Although considerable lowering of male hormone has been associated with decreased libido and decreased ability to achieve spontaneous erections (5, 6), the minimum level of testosterone needed to maintain it is not really known. Further, exogenous testosterone showed an improvement in libido and nocturnal penile tumescence in younger hypogonadal men (7–9) but did not have similar effects in eugonadal men (10).

Organic impotence increases steadily in men more than 50 yr of age and can be multifactorial (11), and a number of these men have been found to have low or low normal androgens. After careful study, when a cause and effect was not found between the low testosterone levels and sexual dysfunction, the researchers (12) suggested that the two conditions should be evaluated separately because they are separate conditions. When the benefit of elevating the slightly low testosterone levels in elderly men was studied by using exogenous testosterone, it raised the hematocrit as well as the prostate-specific antigen level, but positive changes in sexual function were not mentioned (13).

We (14) previously reported a group of men examined for impotence, who, along with mildly low testosterone levels, did not have elevated gonadotropin levels and in whom endogenous testosterone levels could be restored to the normal range by the use of clomiphene citrate. Our current work is designed to study the effect of raising the levels of circulating testosterone without suppressing the hypothalamic-pituitary axis on libido and erectile function. During a recent consensus conference it was decided that such studies were needed in the area of sexual function in aging men (15).

Subjects and Methods

Subject selection

Men were considered candidates for this study when they had the complaint of erectile dysfunction for 6 or more months and low serum free testosterone levels and normal (or unstimulated) serum gonadotropin levels in an early (0800–1000 h) sample. An overlap was permitted in the normal range for serum total testosterone; although the normal range was greater than 250 ng/dL, patients were accepted for study when the level was equal to or less than 275 ng/dL. This was to compensate for the higher sex hormone-binding globulin level seen in older men. All candidates had normal results on magnetic resonance image

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studies of the hypothalamic-pituitary axis. They all had normal levels of serum PRL, estradiol, and sex-hormone binding globulin.

Nocturnal tumescence and rigidity testing

Twenty-five men met the initial criteria, and they were screened with nocturnal penile tumescence and rigidity testing with the portable RigiScan monitor (Dacomed Corp., Minneapolis, MN). The results were automatically documented quantitatively with a software analysis package supplied by Dacomed. Although no clear consensus exists on which measured parameters are best to monitor, we chose what appeared to be the best overall measures of tumescence and rigidity: average maximum rigidity of the tip lead, average maximum tumescence of the base lead, change in tumescence at the base, total area under the curve of the tip lead rigidity, and total area under the curve of base lead tumescence.

Four men (16%) with normal nocturnal tracings were rejected for study because they were proven to have psychological impotence. Of the remaining 21 men who qualified, 2 declined the study, 1 withdrew during the study, and 1 was deleted from the study for not following the protocol. Seventeen men qualified and completed the study. The demographic data are listed in Table 1.

Questionnaires

Patients were asked to fill out detailed questionnaires that included sexual satisfaction assessment, global sexual satisfaction index, and frequency of sexual activity. These questionnaires were completed before the study and after each study phase. The sexual satisfaction assessment is based on scoring 12 statements answered true or false, with 1 point being awarded for each answered true. The total score reflects overall satisfaction, with 12 being the highest and 0, the lowest. The global sexual satisfaction index is a rating scale on how satisfying the sexual relationship is and is rated 0–8, with 8 being "could not be worse." The frequency of sexual activity provides a frequency score as recalled by the patient in being involved in sexual activities on a monthly basis, namely kissing, intercourse, masturbation, and sexual fantasy. A score of actual *vs.* perceived ideal frequency of intercourse provided an assessment of performance over desire.

Stimulation tests

As part of the initial screening, the functional capacity of the pituitary gland was tested with an iv bolus of GnRH (100 μg); LH and FSH were measured at baseline and 30 and 60 min after the injection. The hypothalamus was stimulated with a clomiphene challenge test using clomiphene citrate (50 mg, orally, twice a day for 7 days), and LH, FSH, total testosterone, and free testosterone were measured at baseline and on days 7 and 10.

Drug treatment

During the treatment phase, patients were selected to receive either clomiphene citrate (50 mg) or a placebo on Monday, Wednesday, and Friday by computer randomization in double blinded fashion. Patients were given drug A for 8 weeks and, after a washout of 2 weeks, were given drug B for 8 weeks (clomiphene citrate and an exact matching placebo were supplied by Marion Merrell Dow, Kansas City, MO). During treatment with drugs A and B, serum levels of LH, FSH, total testosterone, and free testosterone were measured on Friday morning at the end of the first and second months within 2 h of taking the last tablet of clomiphene or placebo.

After each drug was given, the patient was asked about sexual function and libido and whether he thought the tablet was the active drug or the placebo. Nocturnal penile testing was also carried out after each drug phase, and the questionnaires were filled out again. Although nocturnal penile tumescence measures the ability to respond, the questionnaires monitored actual performance as well as sexual desires.

Statistical analysis

Challenge tests were analyzed using an analysis of variance with repeated measures (BMDP2V Statistical Software, Los Angeles, CA). Treatment data and RigiScan and questionnaire results were analyzed using one-way analysis of variance. *Post-hoc* testing for significant pairs was performed using the Scheffe's test (BMDP7D Statistical Software). Contingency tables were analyzed using the Fisher exact test or a χ^2 analysis, as appropriate. In all instances, probability is two-tailed, with $P < 0.05$ regarded as statistically significant.

Laboratory testing

Hormonal measurements were made using standard RIA kits in the RIA laboratory at the Lahey Clinic. An exception was the sex hormone-binding globulin assay, which was performed by Nichols Institute (Tarzana, CA).

Of special note were the assays for LH, FSH, total testosterone, and free testosterone. The LH and FSH assays were performed with materials from Becton Dickinson Immunodiagnostics (Orangeburg, NY), in which goat antirabbit antiserum is used. The LH standards are calibrated against the WHO First International Reference Preparation (68/40) of pituitary human LH for immunoassay; the FSH standards are calibrated against the WHO Second International Reference Preparation of human FSH (78/549) in a single antibody system. The calculated sensitivities are 1.0 mIU/mL for LH and 0.3 mIU/mL for FSH. The interassay variations are based on results from lyophilized human serum-based controls. For the LH mean (±SD) of 9.38 ± 0.92 mIU, the coefficient of variation was 9.8%; for the mean of 36.40 ± 1.70 mIU, the coefficient of variation was 4.7%; for the FSH mean of 2.92 ± 0.36 mIU, the coefficient of variation was 12.3%; for the mean of 11.82 ± 0.76 mIU, the coefficient of variation was 6.4%.

The total testosterone analysis was performed with materials from Binax (Portland, ME) in a standard, double antibody RIA method using rabbit (antihuman) testosterone antiserum. The second antibody is goat (antirabbit) γ-globulin. The testosterone standard is a solution of 2000 ng/dL testosterone in a human serum base. The interassay variations are based on results from lyophilized human serum-based controls. For the total testosterone mean of 53.15 ± 4.45 ng/dL, the coefficient of variation was 8.4%; for the mean of 607.08 ± 39.92 ng/dL, the coefficient of variation was 6.6%.

The free testosterone analysis was performed with materials from Diagnostic Products Corp. (Los Angeles, CA). It is a direct, or single tube assay, not calculated as a function of total testosterone and sex hormone-binding globulin, and uses polypropylene tubes coated with rabbit antibodies to free testosterone. The standards are various concentrations of free testosterone (0–50 pg/mL) in human serum. The interassay variations are based on results from lyophilized human serum-based controls. For the free testosterone mean (±SD) of 3.15 ± 0.32 pg/mL, the coefficient of variation was 10.3%; for the mean of 14.53 ± 1.16 pg/mL, the coefficient of variation was 8.07%.

Results

Seventeen men, with a median age of 60.5 yr (range, 42–71 yr), completed the study. The detailed baseline hormone data are listed in Table 2.

GnRH stimulation

Stimulation of pituitary function in the men (n = 17) with GnRH showed a statistically significant rise in LH and FSH at 30 and 60 min. The LH level rose from 6.67 ± 1.53 mIU/mL (±SD) at baseline to 21.61 ± 7.19 mIU/mL at 30 min ($P < 0.001$) and 19.50 ± 6.62 mIU/mL at 60 min ($P < 0.001$) after stimulation. The FSH level rose from 3.22 ± 1.40 mIU/mL (±SD) at baseline to 7.61 ± 3.31 mIU/mL at 30 min ($P < 0.001$) and 7.50 ± 3.13 mIU/mL at 60 min ($P < 0.001$) after stimulation (significance determined by analysis of variance with repeated measures).

TABLE 1. Demographic data on men with sexual dysfunction

Case, age (yr)	Duration (yr)	Type of impotence	Testes (cm)	Stress	Alcohol	Medications	Medical diagnosis
1,61	1.75	Gradual, partial	4.0 × 3.0	0	0–1+	Dipyridamole; diltiazem; aspirin	S/P carcinoma of sigmoid; S/P hip arthroplasty; coronary artery disease; S/P myocardial infarction
2,64	4.5	Gradual, partial	3.5 × 2/5	0	0–1+	Lovastatin	Hyperlipidemia; borderline hypertension
3,71	4.0	Progressive, total	4.5 × 3.5	0	0	Insulin	Type II diabetes mellitus; basal cell carcinoma; S/P colectomy (ulcerative colitis)
4,65	2.0	Progressive, total	4.0 × 3.0	0	2+	Enlalapril; S/P nicotine (10 yr)	Hypertension; chronic obstructive pulmonary disease
5,70	13.5	Progressive, total	4.0 × 3.0	0	0	L-T; gemfibrozil; hydrochlorothiazide; amiloride	Hyperlipidemia; hypothyroidism; hypertension
6,59	3.0	Gradual, partial	5.0 × 4.0 L 4.0 × 3.0 R varicocele	0	1+	Atenolol; lovastatin; S/P nicotine (14 yr)	Hyperlipidemia; hypertension; S/P cerebrovascular accident
7,48	5.0	Gradual, partial	4.0 × 3.0	1+ work	1+	Naproxen	Tendinitis
8,47	10.0	Relative, partial	4.0 × 3.0 L 4.5 × 3.5 R	2+ work, home	1+	S/P Nicotine (5 yr)	Duodenitis; wife, uterine carcinoma
9,68	4.0	Progressive, total	4.0 × 3.0	0	1+	Dipyridamole; lovastatin; S/P glybride	S/P Cerebrovascular accident; hyperlipidemia; type II diabetes mellitus; coronary artery disease (S/P myocardial infarction)
10,42	2.0	Intermittent, ↓ libido	5.0 × 3.5	2+ work, travel	0–1+	Theophyllin; glipizide	Asthma; type II diabetes mellitus
11,63	1.25	Gradual, partial	4.0 × 3.0 L 3.5 × 2.5 R	1+ medical	1+	Digoxin; warfarin; quinidine; furosemide; pindolol	Atrial fibrillation; ventricular tachycardia (AICD): coronary artery disease (S/P coronary artery bypass graft)
12,68	4.0	Progressive, total	5.5 × 4.0 L 5.0 × 3.5 R	0	0–1+	Glyburide; enalipril	Type II diabetes mellitus; hypertension
13,52	0.75	Gradual, partial	4.0 × 3.0 varicocele	0	1+	None	Sclerosing epithelioma; squamous cell carcinoma *in situ*
14,60	3.5	Progressive, total	5.0 × 4.0	1+ work	0	S/P nicotine (3 yr)	Type II diabetes mellitus; silent ischemia
15,60	5.0	Gradual, partial	4.0 × 3.0	0	0	Glyburide; S/P hydrochlorothiazide; allopurinol	Type II diabetes mellitus; hypertension; hyperuricemia
16,56	0.5	Partial, ↓ libido	4.5 × 3.5 Peyronie's disease	1+ financial	0	S/P nicotine (6 yr); nifedipine; diltiazem	S/P carotid stenosis; coronary artery disease (angina)
17,68	10.0	Partial, ↓ libido	3.0 × 2.5	2+ marital	0–1+	Aspirin	Borderline hypertension; osteoarthritis

S/P, Status post; AICD, automatic implantable cardioverter defibrillator.

TABLE 2. Baseline hormones in men with sexual dysfunction

Case no.	Total testosterone (>250 ng/dL)	Free testosterone (<50 yr old, >16 pg/mL; >50 yr old, >11 pg/mL)	LH (<13 mIU/mL)	FSH (<7 mIU/mL)	PRL (<15 ng/mL)	Estradiol (<35 pg/mL)	Sex hormone binding globulin (0.2–1.4 μcg DHT bound/dL)
1	220	10.4	5	3	2	17	0.6
2	215	9.5	4	2	4	24	0.9
3	210	9.7	6	2	2	8	1.0
4	251	11.2	4	1	3	8	1.4
5	252	10.8	6	6	2	10	0.8
6	258	8.9	6	3	2	34	1.2
7	274	15.7	7	2	4	30	0.6
8	272	12.5	6	4	3	20	0.7
9	256	8.6	7	3	2	33	1.0
10	272	14.3	7	3	2	17	0.6
11	198	8.2	7	5	3	18	1.15
12	146	5.4	7	3	2	17	1.4
13	215	8.2	9	2	4	27	1.13
14	275	9.9	7	4	4	17	1.4
15	263	9.5	10	5	6	26	0.52
16	275	11.4	5	4	4	13	1.0
17	253	10.0	6	4	4	18	1.24

Clomiphene challenge test

The integrity of the entire hypothalamic-pituitary-testicular axis was tested with the clomiphene challenge test and showed a significant response. LH rose from 6.41 ± 1.50 mIU/mL on day 0 to 9.23 ± 2.75 mIU/mL on day 7 ($P < 0.001$) and 9.71 ± 2.69 mIU/mL on day 10 ($P < 0.001$) after stimulation. FSH rose from 3.06 ± 1.09 mIU/mL on day 0 to 5.06 ± 2.46 mIU/mL on day 7 ($P < 0.001$) and to 5.82 ± 2.67 mIU/mL on day 10 ($P < 0.001$) after stimulation (significance determined by analysis of variance with repeated measures). Total testosterone rose from 262.2 ± 75.8 ng/dL on day 0 to 389.2 ± 94.9 ng/dL on day 7 ($P < 0.001$) to 480.7 ± 129.7 ng/dL on day 10 ($P < 0.001$) after stimulation (analysis of variance with repeated measures). The free testosterone level rose from 10.0 ± 2.5 pg/mL on day 0 to 13.9 ± 3.9 pg/mL on day 7 ($P < 0.001$) to 16.3 ± 3.8 pg/mL on day 10 ($P < 0.001$) after stimulation (significance determined by analysis of variance with repeated measures).

Treatment phase

The treatment phase showed a significant response for clomiphene over the placebo as well as over the baseline. This was true for all hormones tested after both the first and second months of therapy (Fig. 1). Also, the values for all hormones did not significantly differ between the first and second months of therapy. The serum level of LH rose from 6.4 ± 1.5 to 10.3 ± 3.5 mIU/mL (±SD) at 1 month and 10.2 ± 3.1 mIU/mL at 2 months ($P < 0.01$). The level of serum FSH likewise rose from 3.4 ± 1.4 to 5.6 ± 2.7 mIU/mL at 1 month

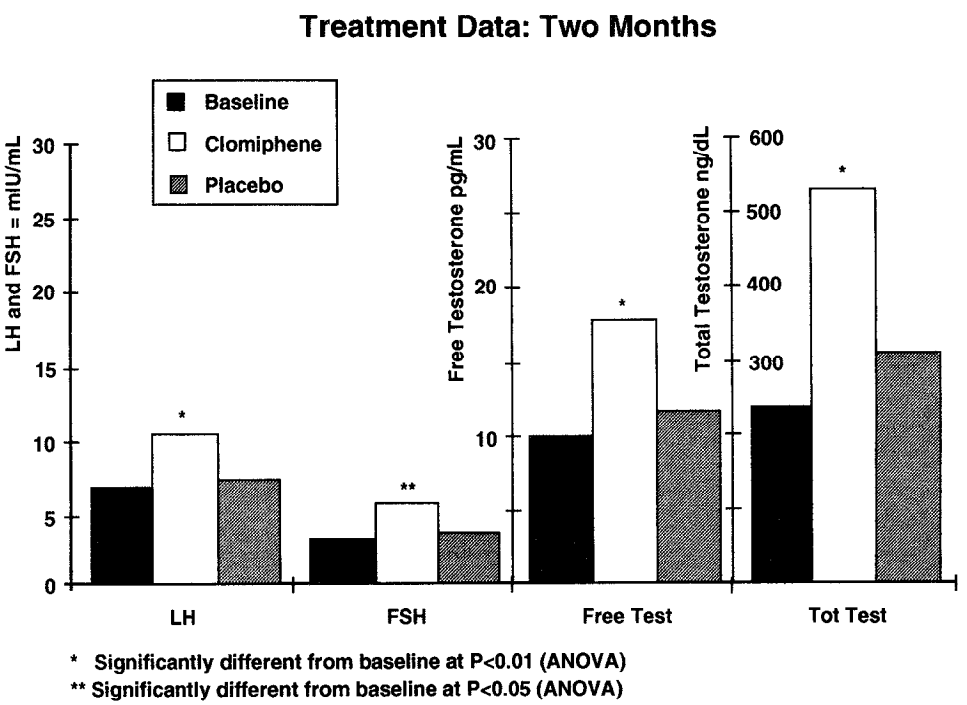

FIG. 1. Hormone levels after 2 months of treatment with either placebo tablets or clomiphene citrate (50 mg, orally) on Monday, Wednesday, and Friday.

TABLE 3. Questionnaire data

Parameter	Baseline	Clomiphene citrate	Placebo	*P* value
Sexual satisfaction index	7.9 ± 3.3	8.8 ± 2.7	8.6 ± 1.9	NS
Global sexual satisfaction index	1.6 ± 1.3	1.6 ± 1.9	1.9 ± 1.7	NS
Kiss (monthly)	4.3 ± 2.7	3.5 ± 2.2	4.0 ± 2.6	NS
Masturbation (monthly)	2.4 ± 1.7	1.8 ± 1.3	2.5 ± 1.8	NS
Intercourse (monthly)	1.9 ± 1.4	2.0 ± 1.4	2.1 ± 1.3	NS
Actual/ideal ratio	0.46 ± 0.35	0.43 ± 0.31	0.44 ± 0.28	NS
Fantasy (monthly)	3.2 ± 2.0	2.8 ± 1.8	3.4 ± 2.2	NS

Values are the mean ± SD.

TABLE 4. Nocturnal tumescence parameters

Parameter	Baseline	Clomiphene citrate	Placebo	*P* value
Total area				
Tip rigidity/h slept, maximum	494.1 ± 710.8	417.4 ± 531.3	301.8 ± 413.1	NS
Average rigidity, tip	15.1 ± 14.1	15.8 ± 19.8	15.2 ± 15.8	NS
Total area base				
Tumescence/h slept, maximum	124.2 ± 95.8	142.9 ± 90.1	94.5 ± 73.2	NS
Average tumescence base	7.6 ± 2.9	7.0 ± 3.4	7.6 ± 2.9	NS
Change in tumescence base	17.8 ± 7.4	19.1 ± 7.5	16.5 ± 7.2	NS

Values are the mean ± SD.

and was at 5.6 ± 3.4 mIU/mL at 2 months ($P < 0.05$). The level of serum total testosterone was also significant, rising from 237.6 ± 38.3 to 549.6 ± 131.9 ng/dL at 1 month and to 527.0 ± 149.9 ng/dL at 2 months ($P < 0.01$). The level of serum free testosterone rose in parallel from 10.0 ± 2.5 to 19.2 ± 3.9 pg/mL at 1 month and 17.8 ± 5.0 pg/mL at 2 months ($P < 0.01$).

Questionnaires and nocturnal monitoring

The responses to questionnaires (Table 3) revealed no significant changes in a variety of parameters related to sexual function as viewed subjectively by the patient. χ^2 analysis of the patients' response, when they were asked whether they thought that drug A or drug B was the active drug, confirmed that the subjects were not able to distinguish correctly. Objective monitoring of nocturnal tumescence and rigidity with the RigiScan also did not show any significant change during any phase of treatment (Table 4).

Secondary analysis

In secondary analysis of the data, when the patients were separated by the median age of the sample, the mean ages of the two groups were 53.0 yr (n = 8) and 66.4 yr (n = 9). Isolated parameters were found that seemed to improve with higher testosterone levels in the younger group. This was true of both nocturnal penile tumescence and rigidity testing as well as from the results of the questionnaires (Table 5).

In a further secondary analysis, the patient population was separated by men with (n = 9) or without (n = 8) diabetes, hypertension, or both (Table 6). There was no significant age difference, nor was there any difference in the basal hormone levels. The men without diabetes and hypertension showed some limited significant sexual improvement in both objective and subjective measurements. The stimulated total testosterone level was not different in the two groups, but the free testosterone level showed a trend toward significance in men without diabetes or hypertension.

TABLE 5. Objective and subjective sexual responses in the patient population separated into younger and older groups during the treatment phase with clomiphene citrate

	Younger	Older	*P* value
No.	8	9	
Mean age (yr)	53.0	66.4	
Baseline total testosterone[a]	263 ± 20	222 ± 36	<0.013[b]
Stimulated total testosterone[a]	489 ± 150	577 ± 145	NS
Baseline free testosterone[a]	10.3 ± 2.7	9.3 ± 1.8	NS
Stimulated free testosterone[a]	17.9 ± 5.6	18.8 ± 4.0	NS
Average tip rigidity[a]	25.9 ± 23.6	6.6 ± 10.2	<0.003[c]
Change base tumescence[a]	21.6 ± 8.05	16.8 ± 6.7	<0.040[c]
Average base tumescence[a]	7.8 ± 3.2	6.3 ± 3.7	<0.059[c]
Sexual satisfaction index[a]	10.0 ± 0.6	7.5 ± 3.6	<0.032[c]
Intercourse	2.7 ± 1.6	1.2 ± 0.4	<0.029[c]
Actual *vs.* ideal	0.6 ± 0.4	0.3 ± 0.1	<0.066[c]

[a] Mean ± SD.
[b] By two-tailed *t* test.
[c] By two-way analysis of variance; significance is consistent across treatment *vs.* placebo groups.

Discussion

Sexual function declines with age, and, as noted by Martin (16), even in healthy men with several partners, the frequency of sexual intercourse declined to nearly nonexistent levels in a large number of men more than 65 yr old. On a similar note, but in a less permissive society, Kinsey and colleagues (17) provided a reason for this decline when they reported an increasing prevalence of impotence with advancing age (<2% until age 40 yr, 6.7% by age 55 yr, and increasing to 24% by age 70 yr). Recently, the community-based random sample of noninstitutionalized men provided similar data, reporting that the prevalence of total impotence tripled from 5% to 15% between 40 and 70 yr of age. More recently, it was thought that as many as 52% of men more than 40 yr of age had some degree of sexual difficulty (18).

A slow but steady decline in the level of circulating androgens is known to occur in aging men. Declining levels of total testosterone (19), free testosterone (4), and bioavailable testosterone (20) have suggested decreasing testicular func-

TABLE 6. Separation of patient population by medical condition, *i.e.* those with and without diabetes or hypertension or both, during the treatment phase with clomiphene citrate

	Without diabetes mellitus/hypertension	With diabetes mellitus/hypertension	*P* value
No.[a]	8	9	
Mean age (yr)	62.6	57.4	NS[b]
Baseline total testosterone[c]	240 ± 37	242 ± 41	NS[b]
Stimulated total testosterone[c]	541 ± 147	531 ± 160	NS[b]
Baseline free testosterone[c]	10.7 ± 2.5	9.8 ± 2.4	NS[b]
Stimulated free testosterone[c]	20.1 ± 5.4	16.3 ± 4.0	<0.11 (NS[b] 0)
Average tip rigidity[c]	21.8 ± 20.7	10.4 ± 18.4	<0.017[d]
Change base tumescence[c]	21.0 ± 8.1	17.3 ± 6.9	<0.006[d]
Masturbation[c]	2.2 ± 1.6	1.3 ± 0.8	<0.006[d]
Global sexual satisfaction index	2.8 ± 2.4	0.86 ± 1.07	<0.018[d]

[a] Two patients had both diabetes and hypertension.
[b] By two-tailed *t* test.
[c] Mean ± SD.
[d] By Two-way analysis of variance; significance is consistent across treatment *vs.* placebo groups.

tion. Rising gonadotropin levels (21, 22), altered gonadotropin rhythm impulses (23–25), and lowered bioavailable/immunoassay ratios of gonadotropins (26) have supported the impact of aging on the hypothalamic-pituitary axis, suggesting that age in men affects the entire hypothalamic-gonadotropin-Leydig cell axis (27).

Armed with the facts of hypogonadism and decrease in sexual activity and with the prior knowledge that sexual behavior and libido are affected by circulating androgens (28–30), causality to the declining androgens for decreased sexual function was assigned by association (16). Closer examination of the relationship between hypogonadism and impotence suggested that although hypogonadism and impotence are commonly associated, they may be independent problems associated with aging (12).

The conclusion found support when reduced androgen levels were restored by exogenously administered testosterone but did not seem to restore sexual capabilities (9, 10, 28). Despite this evidence, testosterone replacement in older men is practiced routinely when a decline in sexual function is reported. These practices found support in the lay literature and may expose elderly men to prostate stimulation and other potentially undesired effects (13).

Secondary hypogonadism, as defined in our patients, refers to a low free testosterone level that is not compensated for by an increase in LH secretion. Our work, reported in this article, helps to nullify the idea of using testosterone indiscriminately in an aging population with the explicit purpose of restoring sexual function. Whereas previous attempts to use exogenous testosterone did not help, we have shown that elevated endogenous testosterone levels did not fare any better. To assess subjective sexual performance, we questioned the patients by using focused questionnaires. To judge objective sexual capability, we used nocturnal penile tumescence and rigidity monitoring and, instead of using exogenous testosterone, which can suppress the hypothalamic-pituitary axis, we used clomiphene citrate in a population of 17 impotent men with secondary hypogonadism. Clomiphene citrate, as we (14) reported previously in this type of population, elevates circulating androgen levels by stimulating the Leydig cells indirectly by means of stimulating GnRH production from the hypothalamus. Despite the significant rise in androgen levels over a moderate period of time (2 months), no clear improvement occurred in the subjects' self-reported sexual behavior or their objective nocturnal penile tumescence and rigidity measurements. Questioning the patients in the office at each stage of the double blind, placebo-controlled trial revealed that the patients were unable to distinguish between the active and inactive drug.

If, however, our treatment group was divided by the median age of the population, the younger men's responses became significant compared with those of the older men. The same was somewhat true when the responses of men without hypertension or diabetes were compared with those of men who had these conditions. Although the small numbers of men prevent sweeping conclusions, further studies would seem warranted. Supporting our observations, Gray *et al.* (31) and Handelsman (32) also showed that older men with ill health had lower testosterone levels than their healthy age-matched counterparts. Lower free testosterone levels were shown in men with hypertension when they were taking several different antihypertensive medications (32, 33). It is also possible, and probable, that many conditions, especially diabetes and hypertension, cause erectile dysfunction without any effect on the hormone levels, because of direct neural and vascular damage.

In certain circumstances, especially in younger patients and those who do not manifest one or more chronic diseases, *i.e.*, diabetes and hypertension, a short course of clomiphene citrate may be tried for several months when the free testosterone level is seen to be lowered. It must also be remembered that 16% of our initial study group, who also had slightly low free testosterone levels, had completely normal results on nocturnal penile tumescence testing consistent with psychological impotence. Thus, the baseline status of the patient must be ascertained accurately either by history or nocturnal tumescence testing.

Although certain selected patients may respond to hormonal augmentation, the clear message seems to be that sexual dysfunction and associated low testosterone levels are not causally related in all patients and that androgens should not be given indiscriminately to every man over a predetermined age.

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Male Hypogonadotropic Hypogonadism: Factors Influencing Response to Human Chorionic Gonadotropin and Human Menopausal Gonadotropin, Including Prior Exogenous Androgens*

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ABSTRACT. Although testosterone (T) therapy is sufficient for maturation and maintenance of secondary sex characteristics in hypogonadal men, gonadotropins are required for stimulation of spermatogenesis. Thirteen men with hypogonadotropic hypogonadism received treatment with hCG, followed in 12 by the addition of human menopausal gonadotropin (hMG). All initially had undetectable serum LH and FSH and low T levels and were azoospermic with small testes. During therapy, all achieved normal male levels of T. Twelve of 13 had marked and continuous increase in testicular volume. Three men had sperm in the ejaculate with hCG treatment alone. All but 1 patient developed sperm in their seminal fluid during combined hCG and hMG therapy. Two men achieved three pregnancies, and 2 more had semen that produced hamster oocyte penetration assays in the fertile range during the protocol period. Four of 5 who achieved sperm densities greater than 1 million/ml while receiving combined therapy maintained or increased sperm production while receiving continued hCG therapy after hMG was withdrawn.

We examined the response to gonadotropin therapy of men who had received previous T therapy and those who had not. There were no differences in rapidity or degree of response, as assessed by rise in serum T, increase in testis volume, or maximal sperm density achieved. Multiple pituitary deficits and cryptorchidism were negative prognostic factors.

In summary, the prognosis for successful stimulation of spermatogenesis in men with hypogonadotropic hypogonadism treated with hCG/hMG is good and not adversely affected by prior androgen treatment. Despite undetectable serum FSH levels, hCG treatment was sufficient to both initiate and maintain spermatogenesis in some patients. (*J Clin Endocrinol Metab* **61:** 746, 1985)

TREATMENT of hypogonadotropic hypogonadism in men is directed at two goals. The first is the restoration of a normal androgen milieu, allowing successful virilization. The second is the induction of fertility. Adult serum testosterone (T) levels may be achieved by either exogenously administered T or stimulation of endogenous T production with hCG. The long-acting T esters have several advantages in this regard. Firstly, they are significantly less expensive than an equally effective dose of hCG. Secondly, normal virilization and potency can be maintained with biweekly or less frequent injections of T enanthate or T cypionate, whereas hCG is usually administered two or three times a week.

When fertility is desired, however, testicular stimulation with gonadotropins is necessary, and exogenous T is no longer sufficient. Several investigators have treated men with hypogonadotropic hypogonadism with gonadotropins (1–6) and LHRH (7–9). We had the opportunity to study a group of hypogonadotropic men prospectively with a standard protocol. This allowed us to assess not only the likelihood of response, but also the importance of several factors that may have prognostic significance.

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Materials and Methods

Patients

During a 2-yr period, we treated 13 gonadotropin-deficient men, 19–42 yr of age, with exogenous gonadotropins (Table 1). This report includes all of these men. Only 7 of the men were married and immediately interested in fathering children. The other 6 were unmarried and participated hoping to obtain information of prognostic value. Eight men had hypogonadotropic eunuchoidism (HE), *i.e.* isolated gonadotropin deficiency which was present before puberty. Six of these 8 had either hyposmia or anosmia. One had associated primary adrenal failure originally appearing at age 3 yr. Two men had isolated gonadotropin deficiency (IGD) which developed after normal

TABLE 1. Hypogonadotropic men: clinical information

Patient	Age (yr)	Diagnosis	Previous androgen therapy (months)	Serum T (ng/dl)	LH/FSH	Testis vol (ml)[a]	Other
A[b]	21	HE		70	u	<1.0	
B	27	HE	96	60	u	2.4	
C[b]	29	HE	96	62	u	2.4	
D[b]	26	HE	60	60	u	1.5	
E[b]	19	HE		20	u	2.0	
F[b]	30	HE		130	u	0.7	Previous orchiopexy for undescended testis
G[c]	28	HE	71	150	u	4.1	
H	23	HE	60	180	u	2.1	Addison's disease, L. inguinal testis, previous R. orchiopexy for undescended testis
I	34	IGD	42	80	u	5.1	Previous orchiopexy for undescended testis
J	34	IGD	2	100	u	7.9	1° hypospadius, hemochromatosis
K	28	Panhypopituitarism	96	38	u	0.6	Undescended testis, now scrotal, varicocele
L	23	Panhypopituitarism/DI	60	60	u	4.7	
M[c]	42	Hypopituitarism/DI	87	50	u	3.1	

u, Undetectable; DI, diabetes insipidus.
[a] Mean of volume of each testes.
[b] Anosmia.
[c] Eighteen-month course of hCG 8 yr previously.

puberty had occurred. The first had fathered 2 children when he was 24 and 26 yr, respectively. Subsequently, at age 27 yr, he developed decreased libido and potency. When evaluated 2 yr later, he was hypogonadal and azoospermic, but had no other abnormalities. The etiology of his deficit remains undetermined. The second patient developed symptoms of hypogonadism and a progressive decrease in ejaculate volume at age 30 yr. A diagnosis of idiopathic hemochromatosis was made, and phlebotomy therapy was begun. Other pituitary deficits were excluded by appropriate endocrine testing in all patients with HE and acquired IGD. Radiological evaluation of the sella turcica was normal in all patients, and both patients with acquired IGD had normal visual fields and head computed axial tomographic scans. Three other patients had other pituitary hormonal deficits (Table 1). Two of the 3 (K and M), had idiopathic hypopituitarism diagnosed in infancy; both had normal skull x-rays and visual fields. The third man (L) developed normally until age 12 yr, when his growth rate declined, and pubertal development ceased; he also developed diabetes insipidus. At age 27 yr, subsequent to the protocol described below, an optic glioma was identified. All 3 were receiving replacement doses of T_4. Two (K and M) required glucocorticoid replacement, and the 2 (L and M) with diabetes insipidus also were treated with arginine vasopressin and chlorpropamide. All 3 men were GH deficient, and patients K and L had received human GH therapy in the past.

Of the 13 men, 9 had been treated previously with androgens for greater than 42 months (Table 1). In addition, 3 of these men had been treated with hCG. Patients G and M had received hCG for 18 months 8 yr previously, and patient H had received 4 separate courses of 6–10 weeks of hCG between age 18 and 21 yr (total, 36 weeks). None of the remaining 10 patients had received exogenous gonadotropin therapy for any prolonged period. Four of the 13 men had had cryptorchid testes. In all but 1, both testes were scrotal before the initiation of our study. Three of the 4 had had orchopexies with simultaneous herniorrhaphies, and the fourth was treated prepubertally with a short course of hCG.

Laboratory evaluations

Serum FSH, LH, and T were measured by RIA (10). Assay sensitivity for LH was either 1.0 mIU Second International Reference Preparation (2nd IRP)/ml or 0.2 mIU First IRP (1st IRP)/ml. For FSH, the sensitivities were either 25 ng/ml LER 907/ml or 0.2 mIU 1st IRP/ml. The normal range for T was 280–1440 ng/dl. All samples for each patient were analyzed in the same assay.

Seminal fluid was collected by masturbation after at least 48 h of abstinence and sent to our laboratory through the mail. All samples were analyzed for volume, sperm concentration, and germ cell morphology. Sperm concentrations greater than 10 million/ml were analyzed with a Coulter counter (Coulter Electronics, Hialeah, FL) (11). When the concentration was lower, a hemocytometer technique was used. Before a sample was defined as azoospermic, 15–20 fields were examined in both a wet drop preparation and a centrifuged, fixed, and stained preparation.

Hamster egg sperm penetration assays were performed in four of the men (A, C, H, and I) (12). The fertile range was greater than 15% penetration.

Protocol

Patients were initially treated with im hCG (Profasi, Serono, Braintree, MA). Twelve received 1500–2000 IU, and 1 (E) received 4000 IU three times a week for 3–24 months. In 12 of the men, human menopausal gonadotropin (hMG; Pergonal, Serono), containing 75 IU FSH and 75 IU LH, then was added to the hCG, and both preparations were given 3 times weekly for an additional 4.5–11 months. At the end of the first course of hCG/hMG, 7 men continued to receive hCG alone for 5–36 months; 4 men then received a second course of hMG plus hCG. The dose of hMG was reduced by 50% during this period in 3 of these men (A, D, and F). All injections were self-administered by the patients at home.

At least three serum samples for LH, FSH, and T determinations were collected at 20-min intervals or on separate days either before therapy was begun or after an interval of no therapy of at least 1 month. Serum T was also measured 1 and 3 months after beginning hCG therapy and again after 3 months of hMG/hCG therapy. Single samples were drawn 4–48 h after the last dose of gonadotropins. Seminal fluid was examined at frequent intervals in all men during a baseline period and throughout therapy.

Testicular dimensions were measured with calipers before treatment and at each clinic visit during therapy. Testicular volume was estimated by the following formula: $V = 4/3\ \pi \left(\frac{a}{2}\right)^2\left(\frac{b}{2}\right)$, where a equals the short testicular axis, and b equals the long testicular axis (in centimeters). Volumes are expressed as the average of the 2 testicles. In 87 normal men, testicular volume calculated in this manner was 17.5 ± 0.7 (±SEM) ml (range, 8.5–47.8 ml) (Ley, S. B., C. A. Paulsen, G. Mozaffarian, M. Higley, and J. M. Leonard, in preparation).

Results

Pretreatment

Serum LH and FSH concentrations were undetectable in all patients. Baseline serum T concentrations (mean of at least three samples) and testicular volume are shown in Table 1. All patients had azoospermia on the first 3–24 seminal fluid specimens obtained, and only 1 man had less than 6 azoospermic samples before the appearance of sperm in his ejaculate.

Four men had testicular biopsies. Two HE patients and one patient with multiple deficits (patient K; Fig. 1) had immature seminiferous tubules and no Leydig cells. The single patient with acquired IGD had very few Leydig cells and only immature germinal elements.

Response to therapy

All 13 men responded to hCG with an increase in serum T into or above the normal range. After 3–5 months of hCG treatment, their serum T values ranged from 530–2490 ng/dl (mean, 866 ng/dl). The addition of hMG to hCG for 3 months further increased serum T in most men, with values ranging from 310–2580 ng/dl (mean, 1156). The values in previously cryptorchid men did not differ, ranging from 930–2580 ng/dl.

Twelve of the 13 men treated with hCG had an increase in testicular volume, as shown in Table 2, before the addition of hMG. The volume increased further in 11 of the 12 men during combined hCG and hMG therapy.

Serial testicular biopsies were obtained from one patient (K) with multiple pituitary deficits before treatment and after therapy with hCG and hMG (Fig. 1). There was both an increase in both seminiferous tubular diameter and the number and maturity of germinal cells.

Of the 13 men, 12 had sperm in their ejaculate after a variable period of therapy (Table 2). In 3 men, all with HE, sperm first appeared after treatment with hCG alone for 5–24 months; the maximal sperm concentrations during hCG therapy were 1.1, 13, and 6.5 million/ml. After hMG was added in 2 of these 3 men, sperm concentrations rose to 16.9 and 80 million/ml, respectively. The other 9 men remained azoospermic until after they had received combined hCG and hMG therapy for 0.5–9 months (mean, 5.0 months). Their maximal counts ranged from 1.0–107 million/ml, with those with multiple pituitary defects having the poorest seminal fluid response to treatment.

Of the 13 men, 7 were married and seeking fertility. Four of the 7 men who desired fertility achieved sperm concentrations greater than one million/ml. Of these, 2 fathered a total of 3 children. In spite of normal sperm penetration tests (24%, 21%, and 35% penetration), a third man (C) has not successfully impregnated his apparently normal wife. The fourth man, who had a maximal sperm count of 3.6 million/ml also was unable to achieve a pregnancy, but his sperm count was above one million/ml for only 4 months before completion of the protocol. Withdrawal of hMG led to a rapid return to azoospermia.

hCG, second course

Five men (A, C, G, I, and J) who achieved sperm counts greater than one million/ml during the therapy described continued to receive hCG alone for 5–31 months. During this period, 4 of the 5 had further significant increase in sperm density. One of these men (J) fathered his second child 28 months after stopping hMG during continued hCG therapy. Patient A, who did not maintain sperm in his ejaculate after hMG was discontinued, was 1 of the 3 men with HE who had originally responded to hCG alone. Regression of spermatogenesis may have been due to poor compliance, as he admitted missing one third to one half of his hCG injections during this latter period.

Two additional men did not have more than one mil-

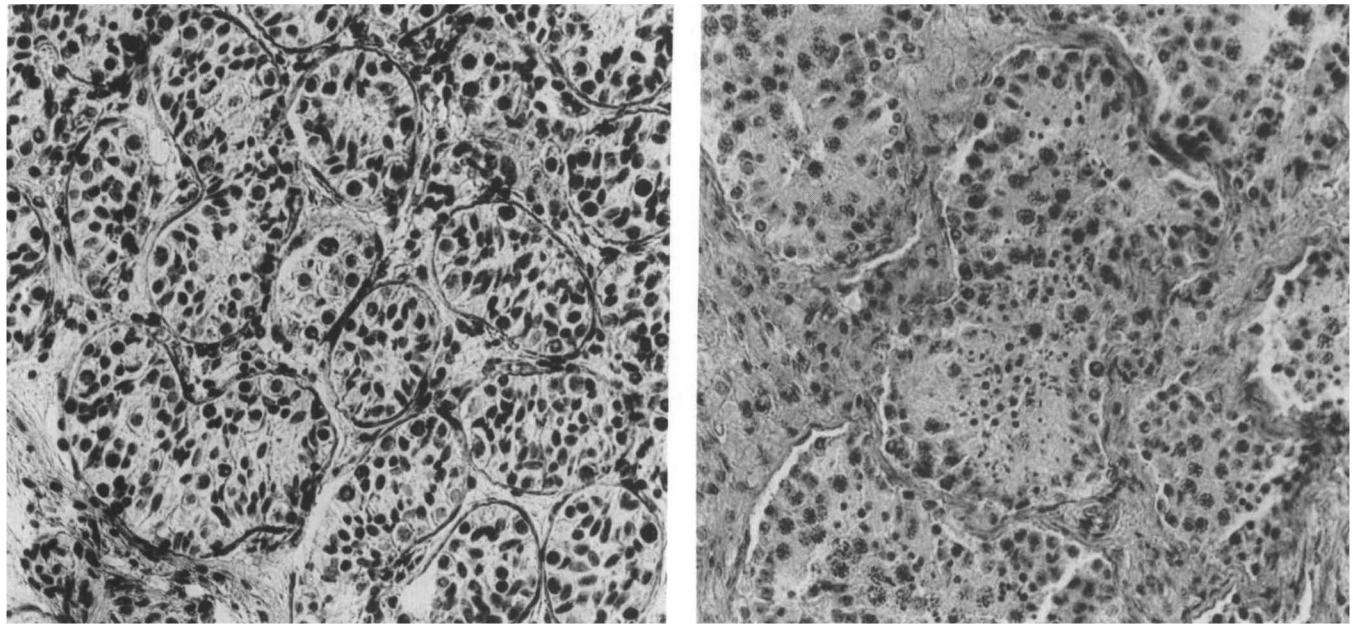

FIG. 1. Testicular biopsies (×64) before (*left*) and during (*right*) treatment with hCG and hMG (patient K). Note the marked increase in the seminiferous tubular diameter, the number and maturity of germinal cells, and interstitial cellularity in response to therapy.

TABLE 2. Hypogonadotropic men: results of treatment

Patient	Therapy (months)			Testis volume			First Spermato-zoa (months)	Max. count (×10⁶/ml)
	hCG	hCG + hMG	Total	Baseline	After hCG	After hCG + hMG		
A[a]	32	4	36	1.0	2.7	3.6	24	16.9
B	13		13	2.4	5.3[b]		5	13.0
C[a]	11	7	18	2.4	7.2	7.7	8	59.0 (123)[c]
D	6	10	16	1.5	4.0	8.4	13	0.2 (2.4)
E	16	11	27	2.0	2.0	2.0	20	5.2
F	7	9	16	0.7	2.3	3.7	13	0.1 (0.1)
G	6	10	16	4.1	4.7	9.0	11	1.3 (3.6)
H	6	9	15	2.1	2.0	3.4		0
I[a,d]	5	9	14	5.1	7.6	9.5	6	7.4 (10.0)
J[d]	3	9	12	7.9	10.4	12.5	4	64.0 (107.0)
K	8	10	18	0.6	2.6	4.9	12	1.0 (0.3)
L	7	10	17	4.7	5.6	6.9	8	1.0
M	6	8	14	3.1	7.0	7.3	12	1.0

[a] Positive sperm penetration assay.
[b] Volume after 1 yr of hCG.
[c] Values in parentheses are the subsequent maximal count obtained with further therapy (see text).
[d] Pregnancy.

lion sperm/ml during the first courses of hCG and hCG/hMG. After discontinuing hMG, they were treated with hCG alone for 5 months. Neither of these men had any further increase in the number of ejaculated sperm.

Finally, patient B, who was not treated with hMG, continued to have sperm densities between 7 and 13 million/ml for 53 months.

hCG and hMG, second course

Four men (A, D, F, and I) were treated with a second course of hCG and hMG. Patient I had a further increase in sperm density, and 5 months after restarting hMG, he impregnated his wife. His maximum sperm count was 10.0 million/ml. Several months later, while treated with hCG alone, he had a positive sperm penetration assay (21% penetration) and a simultaneous density of 7.0 million sperm/ml. A second patient (D) also increased his sperm density during the second course of hMG, and after 8 months, it was 2.4 million/ml. This was a marked increase compared to that during his first course of therapy, but a sperm penetration assay performed at this point was negative. A third patient (A) was described

above. During the second course, he returned to his original level of sperm production, and at a sperm density of 2.5 million/ml, had a clearly positive sperm penetration assay (37% penetration). The fourth man (F) did have an increase in sperm production during the second course of hMG.

Prognostic factors

Previous androgen therapy. We analyzed the response of seven of the eight men with HE (Table 1) to determine the effect of previous androgen therapy on subsequent response to exogenous gonadotropins. The eighth man was excluded because he had bilateral undescended testes and idiopathic Addison's disease. Three of the men had not received any previous therapy and were prepubertal or peripubertal when first examined at 19, 21, and 31 yr of age, respectively. Their initial hormonal therapy was, therefore, the hCG administered in this protocol.

Four of the men had been treated with androgens (T enanthate or T cypionate, 200 mg every 2 weeks) for 60–96 months before beginning treatment with hCG. One of these men had also received hCG for 18 months 8 yr previously, but had since been treated only with T enanthate. These four men were sexually mature, except for small testes, at the time of our study. Comparing Leydig cell response, as measured by serum T levels, after 1 and 3 months of hCG treatment, there was no difference between these two groups (Fig. 2).

Previous androgen therapy also had no negative effect on seminiferous tubular response, as determined by the

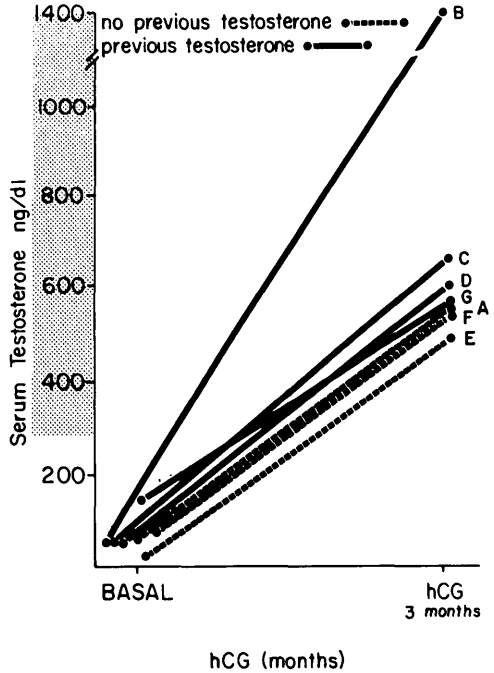

FIG. 2. Serum T responses to hCG in hypogonadotropic hypogonadal men who had (patients B, C, D, and G) and had not (patients A, E, and F) received previous T treatment.

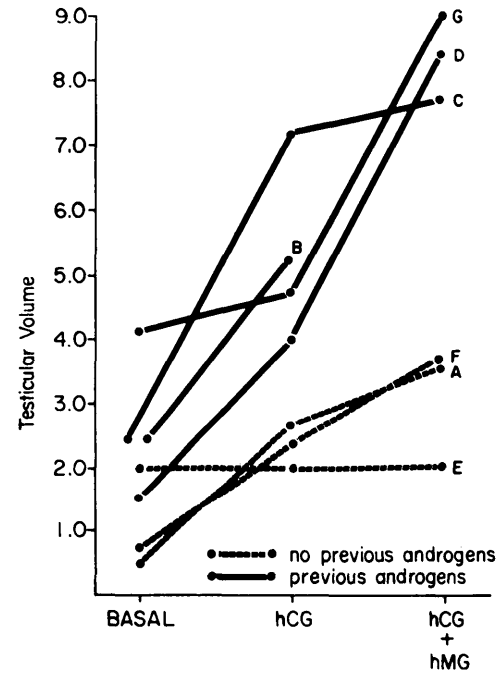

FIG. 3. Changes in testicular volume (milliliters) after therapy with hCG and then hCG and hMG in hypogonadotropic hypogonadal men who had and had not received previous T treatment.

change in testicular volume, the time to appearance of first sperm in the ejaculate, and the maximal sperm count achieved. The changes in testicular volume during therapy are displayed in Fig. 3. The time to appearance of first ejaculated sperm and the maximal count obtained are detailed in Table 2.

Fertility was a goal for only one of the androgen-pretreated men, patient C. He achieved a sperm density greater than 5×10^6/ml. He has so far not had a child, but as stated above, both he and a second androgen-pretreated patient had fertile sperm penetration tests.

Other factors. Men with multiple pituitary defects or cryptorchidism had the least response to treatment (Table 2). Those with postpubertally acquired isolated gonadotropin deficiency had the best response in terms of both sperm density and proven fertility. No other factor, including initial serum T level, initial testis volume, age, or the presence or absence of anosmia, correlated with the likelihood of response.

Toxicity. There were no adverse reactions during the course of drug therapy with either hCG and/or hMG, except for moderate acne in two patients.

Discussion

Patients with hypogonadotropic hypogonadism have been treated with exogenous gonadotropins (1–6) and pulsatile LHRH (7–9). The potential for testicular response to these therapies appears to be good (13). Our

results with 13 consecutive prospectively treated patients confirm these impressions and provide further information on prognostic factors, including prior androgen therapy, which might alter the response to therapy.

Of the 13 hypogonadotropic hypogonadal men treated, administration of hCG normalized serum T in all. After initial treatment with hCG, subsequent addition of hMG led to a further increase in serum T, an effect also found by Sherins *et al.* (6) in 9 HE men. In animals, FSH augments hCG-stimulated T production in hypophysectomized rats (14) and in *in vitro* preparations of rabbit testes (15), an effect perhaps mediated through an increase in Leydig cell LH receptors (16). We found no decrease in T responses in the men with cryptorchidism. Although cryptorchid patients may have compromised testicular steroid synthetic capacity, this difference disappears during prolonged hCG treatment (17).

All but one of our patients had an increase in testes volume during treatment, although testicular volume was not a good predictor of outcome or course of therapy. Final testicular size was low compared to that in normal men (Ley, S. B., C. A. Paulsen, G. Mozaffarian, M. Higley, and J. M. Leonard, in preparation), but 11 of the 13 patients continued to have testicular growth during the study period. All men were initially azoospermic. The only patient who did not develop sperm in his ejaculate during therapy had undescended testes.

Two of the seven married men seeking fertility fathered normal infants. Four unmarried patients also were probably fertile. Two had normal sperm penetration assays, which are highly correlated with male fertility (12), and two more achieved sperm densities of 13 and 5.2 million/ml. It is important to stress that accepted norms for seminal fluid sperm concentration may not be relevant in evaluating fertility in these patients (4). Patient I initiated a pregnancy while sperm concentrations ranged between 6.5 and 10.0 million sperm/ml. He and two other patients also had positive sperm penetration assays when their sperm densities were 7.0, 2.5, and 11.0 million sperm/ml.

Three patients developed sperm during hCG treatment alone, and 2 additional patients had sperm in their ejaculates within 1 month after addition of hMG therapy. In a previous series (6), 11 of 13 men with HE developed mature sperm in the ejaculate within 1 yr of starting hCG as the only therapy; other researchers (18) reported similar patients. hCG alone was also able to sustain spermatogenesis in our patients and in several others reported previously (3, 4, 6, 19). Because the FSH activity of hCG is probably insignificant (<0.001 IU/IU hCG) (20), this response implies that endogenous FSH was present, but in concentrations below current detection limits. This hypothesis is supported by data from Kulin *et al.* (21), who reported a wide variation of urinary gonadotropin values in hypogonadal patients whose serum gonadotropin levels were undetectable. Specifically, in patients with undetectable serum FSH, urinary gonadotropin values were detectable over a 20-fold range. In another report, Boyar *et al.* (22) described 2 patients who had measurable serum LH only during nocturnal spikes. All of our patients had undetectable serum FSH on multiple samples analyzed by RIA. Since those patients who developed sperm in their ejaculate during hCG treatment alone also achieved the highest sperm concentrations, the response to hCG may have prognostic significance.

Our study design allowed us to assess the commonly held impression that prepubertal androgen therapy causes irreversible testicular damage in hypogonadotropic patients. We found no evidence to support this. Initial testis volumes were generally higher in the androgen-pretreated patients. All three men who developed sperm in their ejaculate after hCG therapy alone had previously received T for 2–96 months. Leydig cell potential, as measured by acute and chronic T responses to hCG/hMG, and germ cell responses, as reflected by changes in testicular volume, time to appearance of sperm in the seminal fluid, and maximal sperm concentrations, were not decreased by previous androgen therapy. The lack of effect of androgen pretreatment is supported by other reports in hypogonadal men (3, 23), by the use of Testosterone rebound as a therapy for infertility (24, 25), and by the use of T enanthate as a reversible form of male contraception (26, 27).

Cost and convenience factors favor the T esters, and they are the preferable mode of therapy in hypogonadotropic men until fertility becomes a goal. The major use for either hCG/hMG or pulsatile LHRH is the induction of fertility. Although both therapies are effective, their relative efficacies have not been directly compared. A recent report suggests that response rates are similar (9). LHRH treatment requires that patients wear an infusion pump throughout the day. Because hCG/hMG therapy requires only three or fewer injections a week, gonadotropin therapy is likely to be preferred by many patients. As it is probable that even lower doses of hCG (28, 29) and hMG (6) may be effective, further studies designed to optimize dose and injection schedules may further increase this advantage.

In summary, hypogonadotropic men can be treated effectively and safely with T esters until they desire fertility. We then recommend hCG alone; an early response to this medication probably has prognostic value. From our results and those of others, it seems prudent to treat with hCG alone for 1 yr, or until testicular volume reaches a plateau. Then hMG should be added. Cryptorchidism and prepubertally acquired multiple pituitary defects were negative prognostic factors, whereas

patients with gonadotropin deficiency acquired postpubertally responded quickly and dramatically. Androgen pretreatment did not have a negative effect on outcome. As long as testicular volume continues to increase, therapy should be continued. In some patients, induction of fertility may occur in a matter of months. In others, increased sperm counts may not be obtained for 1–2 yr or longer.

Acknowledgment

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Single *Versus* Repeated Dose Human Chorionic Gonadotropin Stimulation in the Differential Diagnosis of Hypogonadotropic Hypogonadism*

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ABSTRACT. The responses of serum testosterone (T), 17α-hydroxyprogesterone, and 17β-estradiol (E_2) to four im injections of hCG (5000 IU/1.7 m^2) given on days 0, 4, 7, and 10 were studied in 10 prepubertal and 10 pubertal boys with hypogonadotropic hypogonadism (groups O and P, respectively). Serum was obtained before each injection and on day 14. The results were compared with those of controls, 16 prepubertal boys with incomplete testicular descent and 6 pubertal boys with constitutional delay of puberty. Serum T levels increased significantly in groups O and P to 2.0 and 4.6 nmol/liter, respectively, after the first injection, then progressively to 5.8 and 11.2 nmol/liter. Basal T levels of group O did not differ from those of the controls, but were subnormal for group P ($P < 0.001$). Stimulated T levels were subnormal in both groups ($P < 0.01$ and $P < 0.001$), but repeated doses increased the difference from the control value only in group P. A difference in E_2 response between patients and controls appeared in puberty; only the pubertal control boys had substantial increases in E_2 ($P < 0.001$). Our results show that the optimal protocol for a diagnostic hCG test in prepubertal boys is a single dose of hCG, with determination of T levels 4 days later. In puberty, if the basal T levels are inconclusive, repeated doses of hCG should be given with determination of both T and E_2. These findings also suggest that the full inhibitory effect of E_2 on T synthesis results from a pubertal maturation process, possibly induced by endogenous gonadotropins, which cannot be induced by two weeks of hCG stimulation in prepubertal boys or those with hypogonadotropic hypogonadism. (*J Clin Endocrinol Metab* **60:** 333, 1985)

THE DIAGNOSIS of hypogonadotropic hypogonadism (HH) remains difficult despite the improvements brought about by GnRH and TRH tests (1). These tests give false negative results in some boys with HH (1). As serum testosterone (T) responses to hCG are often subnormal in these boys (2–6), combining the hCG test with the GnRH or TRH test might improve the diagnostic accuracy. Before exploring this possibility, however, the sensitivity of the hCG test in the diagnosis of HH needs to be established.

Several different protocols have been proposed for diagnostic hCG stimulation in boys (7–15). All but one (9) involve massive doses given repeatedly, and all use serum T levels as the only response indicator. While such stimulation is probably necessary for proving the absence of Leydig cells, it may not be ideal for differentiating HH from constitutional delay of puberty.

A single dose of hCG causes Leydig cell steroidogenic desensitization in adult rats and men (16–23). This is linked with a decrease in 17α-hydroxylase and 17,20-lyase activity, which, in turn, leads to an accumulation of progesterone and 17α-hydroxyprogesterone (17-OHP) (17, 18). This process is thought to be mediated by estradiol (E_2) through the sequence of gonadotropin-induced aromatase stimulation, increase in E_2 and its occupation of estrogen receptors in Leydig cells (18, 20, 24, 25). An LH-priming effect appears necessary for this enzyme inhibition, as evidenced by the lack of E_2 and 17-OHP responses to a single dose of hCG or LH in prepubertal boys (26) and adult men with HH (27, 28). Prolonged hCG stimulation may, however, cause enzyme inhibition in HH, thus impairing the diagnostic value of the test.

The aim of this study was 1) to determine the kinetics of the T response to single and repeated doses of hCG in boys with HH, normal boys, and boys with constitutional delay of puberty to obtain the simplest, most reliable stimulation protocol for differentiating among them, and 2) to determine if serum E_2 and/or 17-OHP responses gave any further diagnostic information.

Materials and Methods

Subjects

Twenty boys with HH were studied (Table 1). Ten were prepubertal (group O), and 10 had entered puberty (group P)

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TABLE 1. Clinical data of the patients

Patient no.	Age (yr)	Testis vol (ml)[a]	Pubertal stage[b]		Etiology	Deficiency of			
			P	G		GH	TSH	ACTH	ADH
1	7.4		1	1	Prader-Willi syndrome	—	—	—	—
2	8.8		1	1	Prader-Willi syndrome	—	—	—	—
3	11.4		1	1	Breech delivery	+	+	—	—
4	12.0	2.2	1	1	CRF[c]	+	+	+	+
5	12.1	4.0	1	1	CRF	+	+	+	+
6	12.5	0.8	1	1	Histiocytosis-X	+	+	+	+
7	12.8	0.3	1	1	Idiopathic	+	+	+	+
8	13.9	1.4	1	1	Idiopathic	—	—	—	—
9	14.3	0.4	2	1	Kallmann syndrome	—	—	—	—
10	14.4	0.8	1	1	Idiopathic	+	+	+	+
11	15.6	2.8	5	4	Kallmann syndrome	—	—	—	—
12	17.5	2.5	4	4	Pituitary adenoma operated + radiated	+	+	+	—
13	18.5	3.3	5	4	CRF	+	+	+	+
14	19.3	16.7	3	3	CRF	+	+	+	+
15	19.4	3.0	4	4	CRF	+	+	+	+
16	19.4		4	4	CRF	+	+	+	+
17	19.9	3.6	3	3	CRF	+	+	—	—
18	20.5	2.8	3	5	CRF	+	+	+	+
19	22.2	2.4	3	4	CRF	+	+	+	+
20	27.7	9.8	3	4	CRF	+	+	+	+

—, Absent; +, present.
[a] According to Hansen (29).
[b] Pubic hair (P) and genital (G) (excluding testis size) stage (30).
[c] Craniopharyngioma.

during androgen replacement therapy. Five had isolated gonadotropin deficiency; the rest had other pituitary deficiencies as well. Appropriate substitution therapy was given before and during the hCG tests (2 mg GH, 2 or 3 times weekly; T_4 to maintain normal serum T_4 levels; and 10–15 mg/m^2 cortisol in three daily doses). The diagnosis of gonadotropin deficiency was established earlier, and the present data were not used diagnostically. The diagnostic criteria of HH were a clearly prepubertal or subnormal testis size for bone age (29) and absence of pubertal penis growth at bone age 13 yr or older (before any androgen therapy). All patients were followed for several years to confirm deficient testicular growth. In 2 prepubertal boys (patients 1 and 2), the diagnosis was based on subnormal gonadotropin responses to GnRH. All patients with craniopharyngioma were investigated after surgery. The gonadotropin responses to GnRH and the steroidogenic response to hCG were similar in the patients with organic hypopituitarism and the other patients. Thus, the results of all patients with HH were pooled.

All of the pubertal boys had received T enanthate (Primoteston depot, Schering AG, Berlin, West Germany; 1–5 mg/kg (maximum, 250 mg), at 4-week intervals). This therapy was discontinued at least 3 months before the hCG tests.

Previous results from 16 prepubertal boys, aged 1.1–12.4 yr, with suspected incomplete testicular descent (true, or retractile testes) and 6 pubertal boys (genital stage 3) (30), aged 13.9–17.4 yr, with constitutional delay of puberty served as control values (31).

Protocol

Patients and control subjects were given four im injections of hCG (5000 IU/1.7 m^2 each; Pregnyl, Organon, Oss, The Netherlands) on days 0, 4, 7, and 10. Blood for the determination of 17-OHP, T, and E_2 was obtained immediately before each injection and 4 days after the last one.

Methods

The sera were stored at −20 C until analyzed. They were quantified by RIA after chromatography on Lipidex-5000 (17-OHP and T) (32) or Sephadex L-20 (E_2) (33). Samples from an individual subject were analyzed at the same time.

Statistics

Group O was compared with the prepubertal controls, and group P was compared with the pubertal controls. The data were analyzed by BMDP computer programs (34). The means were compared by *t* test for independent and dependent samples (program 3D) and by general univariate and multivariate analysis of variance (program 4V). Because of positive skewness of the distributions, all calculations were made after logarithmic transformation. The specificity and sensitivity of each variable were calculated by applying Bayes' theorem, where sensitivity = (number of true positives)/(number of true positives + false negatives), and specificity = (number of true negatives)/(number of true negatives + false positives). In this study the sensitivity indicates the proportion of correct subnormal findings from all findings in the boys with HH and the specificity the proportion of normal findings from all findings in the controls.

Concentrations in nanomoles per liter (picomoles per liter)

can be converted to nanograms per dl (picograms per ml) by multiplying by 28.8 (T), 33.0 (17-OHP), or 0.27 (E_2).

Results

Steroidogenic response to hCG in boys with HH (Fig. 1)

The mean basal T levels were 0.2 and 0.7 nmol/liter ($P < 0.01$) for groups O and P, respectively. The levels rose to 2.0 and 4.6 nmol/liter after the first injection, then progressively after each injection to 5.8 and 11.2 nmol/liter at the end of the stimulation ($P < 0.001$ and $P < 0.01$ compared with day 4). There was no significant difference between the groups at any time.

E_2 levels did not increase in group O, but were slightly elevated in group P at the end of the stimulation. 17-OHP levels increased gradually, finally reaching a level significantly above the basal. There was no significant difference between the groups basally or after stimulation in either 17-OHP or E_2 levels.

Difference between patients and controls (Fig. 1)

The mean basal T concentration in group O did not differ from that in the controls, but the group P value did. Mean stimulated T levels in both groups were markedly below those in controls. Mean basal E_2 levels in the patients were no different from those in the controls. However, neither group of patients had an E_2 response, whereas the pubertal controls did have an E_2 response. Thus, a difference between the patients and the controls appeared at puberty, and increased throughout the 2-week period of stimulation. The basal 17-OHP levels were markedly different between patients and controls in group P, but after stimulation, the difference was less or disappeared.

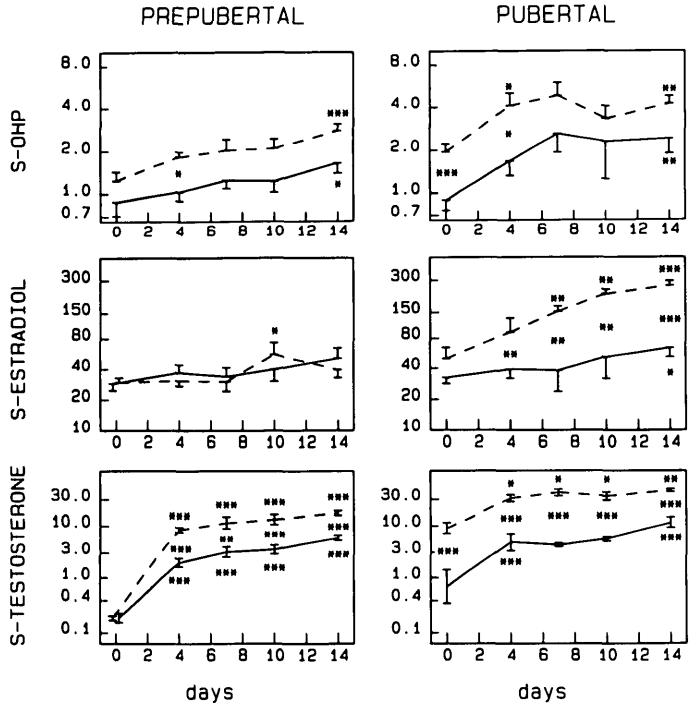

FIG. 1. Effect of four doses of hCG given on days 0, 4, 7, and 10 on serum (S-) concentrations (mean ± SEM) of T (nanomoles per liter), E_2 (picomoles per liter), and 17-OHP (OHP; nanomoles per liter) in prepubertal and pubertal boys with HH (——). The results are compared with those of 16 prepubertal and 6 pubertal (genital stage 3) control subjects (---) studied with the same protocol. Statistically significant differences are indicated, with *asterisks between the curves* for differences between patients and controls, *above the curves* for differences from the basal level in the controls, and *below the curves* for differences from the basal level in the patients. For converting nanomoles per liter (picomoles per liter) to nanograms per dl (picograms per ml), multiply by 28.8 (T), 33.0 (17-OHP), or 0.27 (E_2). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Discriminatory power of different test variables (Fig. 2)

In prepuberty, no HH patient could be identified by the basal T level. By contrast, a stimulated T level was a very specific discriminator and was equally sensitive on days 4 and 14. The E_2 levels had no discriminatory power.

At puberty, the basal T level was a very specific and sensitive discriminator. Stimulation increased the specificity of T on both day 4 and 14. However, for sensitivity, stimulated T exceeded basal T only on day 14. Almost as sensitive as the stimulated T levels on day 14 were the E_2 levels on days 7, 10, and 14.

Discussion

These results clearly demonstrate that the hCG test is a very sensitive discriminator between boys with HH and normal prepubertal boys and between boys with HH and boys with constitutional delay of puberty. For our

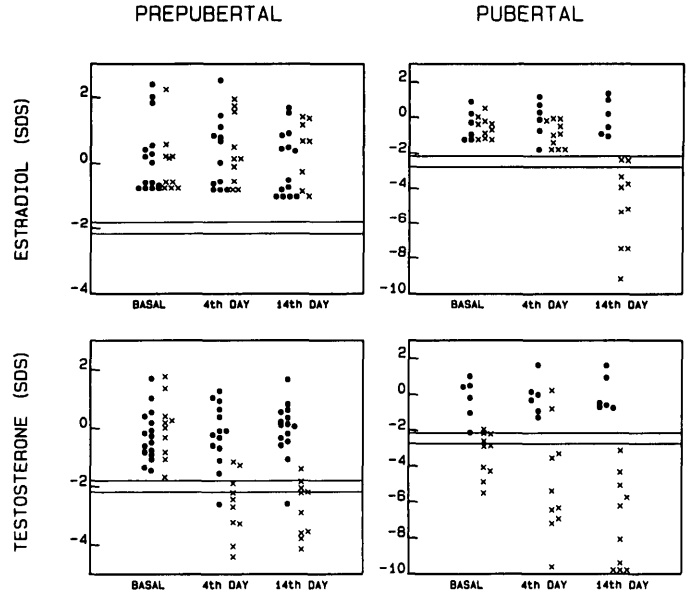

FIG. 2. Discrimination of hypogonadotropic boys (X) from the control subjects (●) by SD scores (SDS) of serum T and E_2 concentrations basally, after the first dose of hCG (4th day), and at the end of stimulation (14th day) in prepubertal (*left panel*) and pubertal (*right panel*) boys. The *two horizontal lines* represent the lower limit of the 90% and 95% confidence ranges, respectively.

prepubertal controls, we could only use boys with incomplete testicular descent, and some of them may have had partial LH deficiency (35). However, if the hCG test differentiated between this control group and boys with unequivocal HH, it should even better distinguish the normal state from HH. Previous studies of short hCG stimulation tests in boys with HH showed the T response to be blunted (2–6). In these studies, the stimulation protocol varied as to dose, number of injections, and interval between doses as well as timing of blood sampling. Thus, the diagnostic significance of the hCG test has not been well established. In the present study, we focused on the kinetics of the steroidogenic response to establish a protocol for this specific purpose. The hCG test is an indirect indicator of gonadotropin deficiency. Hence, for a diagnosis of HH, additional tests (GnRH or TRH) (1) as well as clinical criteria are necessary to exclude primary hypogonadism.

A subnormal T response and little or no 17-OHP and E_2 responses were characteristic of the boys with HH. Similar results were reported in adult men with HH after a short infusion of LH (28) or a single injection of hCG (27). It has been suggested that the acquisition of E_2-synthetizing capacity is a part of pubertal maturation (26), possibly induced by an increase in gonadotropin secretion. This suggestion is supported by our finding of identical E_2 responses in both the boys with HH and the prepubertal controls.

In the prepubertal patients and controls, serum T concentrations increased progressively after repeated hCG injections. This occurred despite an increase in serum 17-OHP levels, which is believed to reflect E_2-mediated inhibition of 17,20-lyase. Thus, it appears that the full inhibitory effect of E_2 on T synthesis only develops with puberty and cannot be induced in prepuberty even by 2 weeks of hCG stimulation. The pubertal patients had a somewhat greater E_2 response and a greater 17-OHP response, presumably as a result of the higher E_2 level.

In prepuberty, a single dose of hCG increased serum T concentrations significantly in both patients and controls. The difference between them was very significant after the first dose, but did not increase with further stimulation. Neither could the specificity or the sensitivity of the stimulated serum T level be increased by longer stimulation. Thus, for prepubertal boys, administration of a single im dose of 5000 IU/1.7 m^2 with determination of the serum T concentration 4 days after the injection gives information similar in value to that produced by a protocol including repeated doses. For pubertal boys, however, if the basal T measurement is inconclusive, longer stimulation with four injections at 3- to 4-day intervals, with determination of both T and E_2 concentrations 4 days after the last injection, appears best.

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Testicular Responsiveness to Chronic Human Chorionic Gonadotropin Administration in Hypogonadotropic Hypogonadism

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ABSTRACT. Steroidogenic responsiveness to long term hCG administration (1500 U three times a week for 23 months) was characterized in 8 males with hypogonadotropic hypogonadism (HH). During hCG treatment, testosterone (T), which was in the prepuberal range under basal conditions, rose considerably to the upper end of the normal range and remained at that level during the 23 months of observation. A 2.5-fold increase was observed in serum levels of 17β-estradiol (E_2) an increment less than seen with T. The increment in 17α-hydroxyprogesterone was also lower than that in T throughout the study; thus, the 17α-hydroxyprogesterone to T ratio, despite continuous hCG administration, remained low. Serum androstenedione was slightly increased during hCG therapy. No significant changes were observed in serum levels of dehydroepiandrosterone. These data indicate that continuous long term hCG administration stimulated T levels in HH, with a relatively small change in E_2. The kinetics of the T and E_2 responses to 2000 U hCG, evaluated after 23 months of therapy, indicated that the testicular response was markedly reduced. No increment in T levels was observed at 24 h; the maximal response occurred at 48 h. This pattern of T response supports the idea that partial testicular desensitization occurs in HH patients receiving chronic treatment with hCG. (*J Clin Endocrinol Metab* **55:** 76, 1982)

hCG PREPARATIONS have been used extensively in patients with hypogonadotropic hypogonadism (HH) as exogenous replacement for gonadotropin deficiency. The clinical improvement which accompanies such treatment indicates that testicular steroidogenesis in HH is well preserved and responds normally to hCG stimulation. However, steroidogenic responsiveness to exogenous hCG administration in HH has not been extensively evaluated. Two recent reports postulate a qualitative and quantitative difference in the acute steroidogenic response to hCG compared to normals (1, 2).

Besides increasing androgens, the administration of pharmacological doses of hCG resulted in paradoxically large increases in estrogens and C-21 androgen precursors in men [progesterone and 17α-hydroxyprogesterone (17OHP)] (3, 4). In animals, hCG administration reduced the specific receptors in the membranes of Leydig cells (5, 6). Estrogen increases and decreases in specific receptors in Leydig cells are well established as side effects of hCG administration. The practical implication of such recent findings during this therapy in humans is still unknown, since few studies have been made on the testicular effects of prolonged use.

We undertook our study with the aim of defining the effects of chronic hCG treatment on C-21, C-19, and C-18 steroids and on the dynamics of the testosterone (T) and 17β-estradiol (E_2) responses to hCG challenge in HH.

Materials and Methods

Eight patients with selective HH, aged 18–31 yr, were studied. All subjects had eunuchoid habitus, prepubertal testicular volume (Table 1), and T blood concentrations within the prepuberal range (Fig. 1). None had hyposmia or cryptorchidism. Family history revealed that their diseases represented isolated sporadic occurrences. The patients were given hCG (Profasi, Serono, Rome, Italy) for correction of androgen deficiency symptoms (1500 U three times a week) for almost 2 yr. Hormonal levels were measured before (basal) and at 4, 8, 16, and 23 months during hCG administration. During therapy, blood was sampled 48 h after drug injection. Previous medication, as reported in Table 1, had been omitted for at least 3 months before this study began (Table 1).

To evaluate the dynamics of T and E_2 responses during chronic hCG administration, 2000 U hCG were given to five patients 48 h after their last injection of 1500 U hCG at the end of replacement therapy. Blood samples were collected daily

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TABLE 1. Clinical details and basal LH and FSH concentrations (mean ± SE) in our patients

Case No.	Age (yr)	Duration of previous therapy (months)	Duration of hormone therapy withdrawal before study (months)	Testis size (ml)		LH (mUI/ml)[a]	FSH (mUI/ml)[b]
				Right	Left		
1	20	6 (hCG)	9	4	3	1.2 ± 0.2	1.2 ± 0.1
2	31	7 (hCG + hMG)	3	2	2	1.9 ± 0.1	1.12 ± 0.08
3	26	24 (hCG)	3	3	3	0.7 ± 0.3	2.5 ± 0.15
4	19	[c]	[c]	3	3-4	1.7 ± 0.1	0.9 ± 0.2
5	18	[c]	[c]	6	6-8	1 ± 0.4	0.62 ± 0.2
6	18	[c]	[c]	2	2-3	1 ± 0.5	1.57 ± 0.16
7	18	19 (hCG + hMG)	3	6	5	2.5 ± 0.1	1 ± 0.09
8	18	24 (hCG + hMG)	12	5-6	5-6	3.1 ± 0.2	0.8 ± 0.13

hMG, Human menopausal gonadotropin.
[a] Normal value in healthy adults, 2.8 ± 0.2.
[b] Normal value in healthy adults, 3.6 ± 0.2.
[c] No therapy.

during the next 6 days. In these subjects, due to the limited serum available, other hormones were not measured.

Plasma T, 17OHP, and androstenedione (A) were measured by RIA using paper chromatography (7, 8) (intraassay coefficients of variations were 11% or less for each of these assays, and interassay variation was 16% or less). Plasma E_2 was evaluated by RIA using specific antiserum after purification on a Sephadex LH-20 column. Coefficients for intra- and interassay variations were 8% and 16%, respectively. Dehydroepiandrosterone (DHEA) was measured in ether-extracted samples using a specific antiserum. Intra- and interassay coefficients were 11.1% and 12.5%, respectively. In both the chronic and acute studies, all samples from each subject were measured in the same assay. Differences were statistically evaluated by analysis of variance. Results in the text and figures are expressed as the mean ±SE.

Results

The mean (±SE) basal T concentration in HH subjects was in the prepuberal range (45 ± 8 ng/dl); E_2 levels were also below normal (4.4 ± 0.4 *vs.* 12.8 ± 1 pg/ml in controls; $P < 0.01$; Fig. 1). The mean basal 17OHP value was 52.6 ± 18.4 (SE) ng/dl, not statistically different from that of eugonadal men (107 ± 11 ng/dl). In four of eight patients, basal 170HP values were, as expected, very low (28.4 ± 6.7 ng/dl). Basal A was 83.9 ± 13 (SE) *vs.* 121 ± 95 ng/dl in normals, and DHEA levels were within the normal range (Fig. 1).

Effect of chronic hCG administration on plasma T and E_2 levels

After 4 months of hCG treatment, blood concentrations of T had increased considerably (11-fold; $P < 0.01$) and were already within the normal range (Fig. 1). A further consistent increase was noted after 8 months of therapy (16-fold above the basal value). Subsequent blood concentrations remained similar to those observed after 8 months of treatment. After an initial 2.5-fold rise in E_2 levels ($P < 0.01$) during the first 4 months, no further marked changes were noted (Fig. 1) in plasma E_2. The magnitudine of the rise was thus much greater for T than for E_2.

Effect on DHEA, A, and 170HP levels

No significant variations above basal values were observed in DHEA levels (Fig. 1). The levels of A were slightly increased, achieving mean values 1.2-, 1.3-, and 1.6-fold above basal levels at 8, 16, and 23 months, respectively. The mean 170HP levels after hCG administration were higher than the basal value (Fig. 1), but the differences became significant from 8 months on ($P < 0.02$). Since the 170HP increase was much lower than the T rise, the 170HP to T ratios 4, 8, 16, and 23 months after hCG treatment were very low (mean ± SE, 0.13 ± 0.02, 0.14 ± 0.02, 0.21 ± 0.01, and 0.16 ± 0.01, respectively), which suggests that no accumulation of C-21 precursors occurred. Interestingly, the relative steroid ratios did not change during the long period of study, hence demonstrating that no steroidogenic variations took place with increased time of treatment

Acute responses of T and E_2 to hCG during chronic hCG therapy

The dynamics of T and E_2 responsiveness were best seen during the period of frequent sampling after an acute administration of 2000 U hCG, 48 h after the last chronic dose of hCG. Figure 2 shows clearly that T levels remained virtually unchanged after 24 h and rose at 48 h, when they were slightly higher than those observed during chronic therapy (1.1 times the basal value). In two subjects, no changes were observed in serum T after the

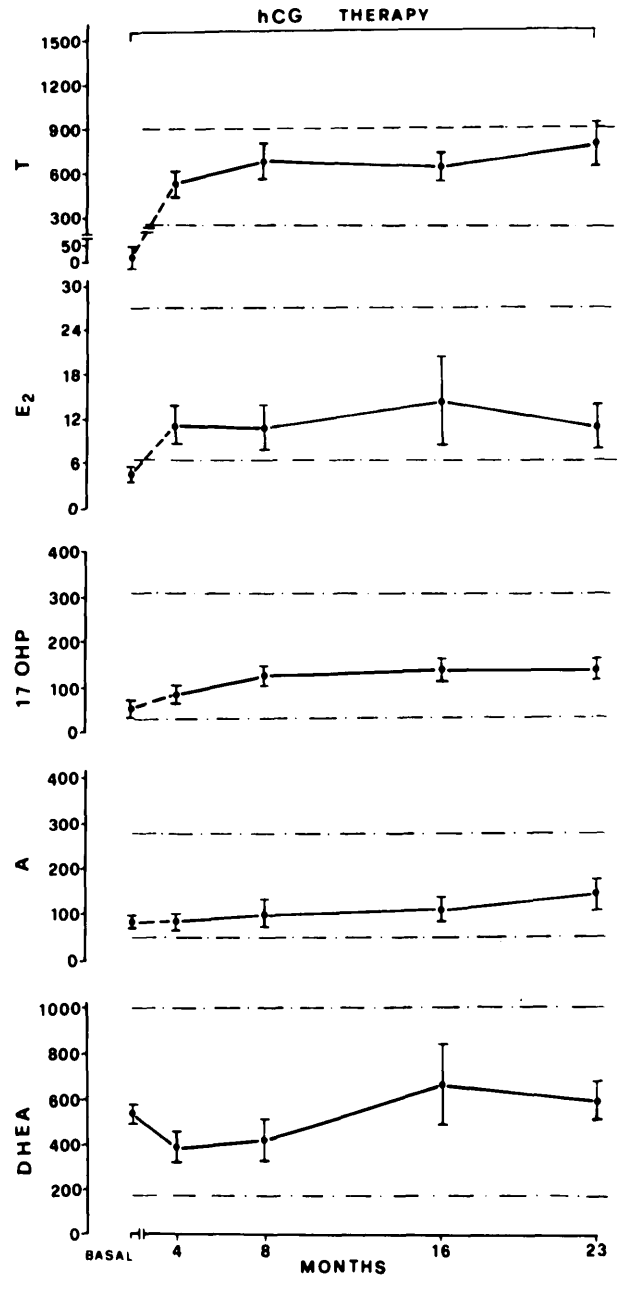

FIG. 1. Serum blood concentrations of T (nanograms per dl), E_2 (picograms per ml), 170HP (nanograms per dl), A (nanograms per dl), and DHEA (nanograms per dl) before (basal) and during long term hCG therapy in our patients. All values are the mean ± SEM. The *dashed lines* encompass the normal range.

2000-U dose of hCG. After 48 h, T levels gradually decreased to a nadir of 300 ± 204 (SE) ng/100 ml at 144 h, the lower limit of our normal values. Hence, the pattern of testicular responsiveness to 2000 U hCG indicates a reduced response of the steroid to the trophic stimulus, with a delayed T rise.

The response peak for E_2 was observed within 24 h, and the E_2 levels tended to plateau for the next 2 days, followed by a fall in blood levels, with a nadir at 144 h (mean ± SE, 5.4 ± 1.9 pg/ml).

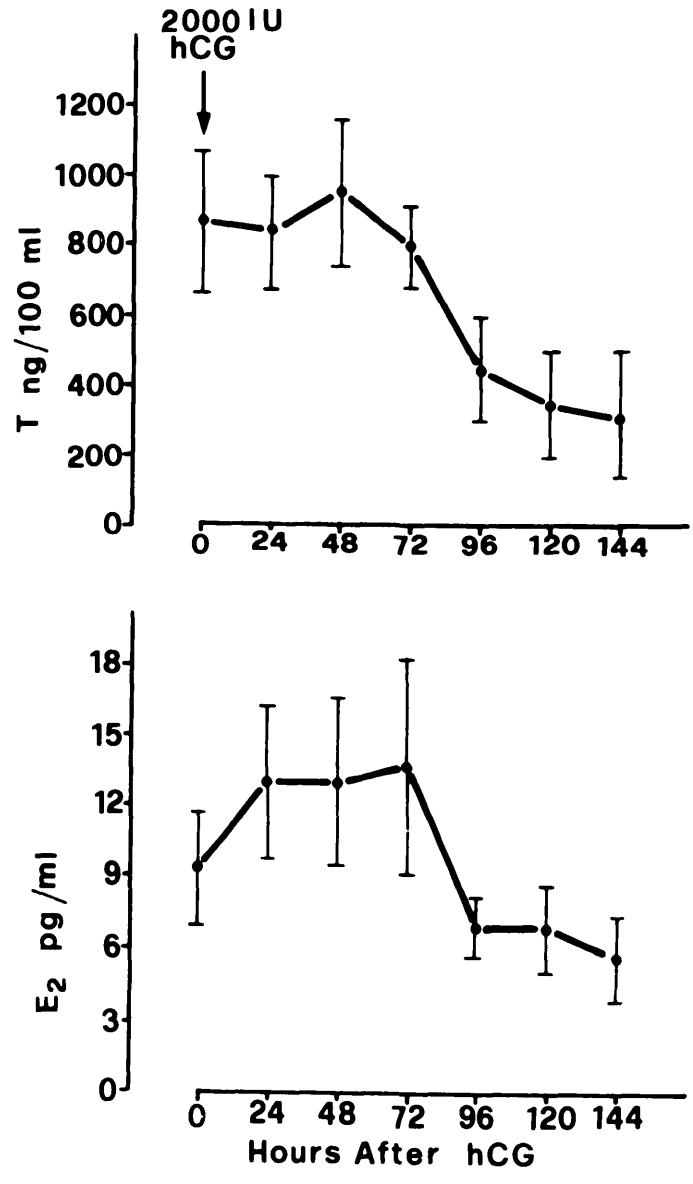

FIG. 2. Kinetics of T and E_2 responses to 2000 U hCG in five hypogonadotropic patients. All values are the mean ± SE.

Discussion

This report shows that hCG chronically administered to HH patients brought about a predominant increase in T and minor increases in E_2 levels. It is noteworthy that after the initial rises observed at 4 and 8 months of therapy with hCG, serum T remained at a plateau despite continuous hCG administration. The hCG course also augmented the serum 170HP and A concentrations. Compared to the T increment, the rises of Δ^4 precursors (170HP and A) were less significant, resulting in low precursor to T ratios. This steroidogenic responsiveness pattern is quite different from that found in normal males.

In normal subjects, an acute load with hCG/human LH resulted in an exaggerated increase in E_2, with a

relatively smaller rise in T (3, 4). The stimulation of aromatase enzyme activity by hCG (9, 10) may account for this marked increase in estrogen. This is commonly explained as resulting in a relative temporary defect in T biosynthesis, on the one hand, and an accumulation of precursors via suppression of some enzymes involved in its biosynthesis, mainly 17α-hydroxylase, 17,20 desmolase, or 17β-dehydrogenase activities, on the other (desensitization) (11–14). Interestingly, a recent report shows the persistence of this block for 10 days after acute hCG administration (15).

Therapy with hCG increased E_2 levels from below normal to normal levels. hCG stimulates estrogen production not only by directly increasing the aromatase activity in the testis (9, 10), but also by raising the level of T, which is then converted to E_2. This latter effect appears more likely to account for the hCG stimulation of E_2 production seen in HH subjects and suggests that long term hCG administration in HH does not inappropriately enhance aromatase activity. This either implies that hCG does not stimulate aromatase activity as it does in normal subjects or that much higher amounts of hormone are necessary in HH.

The present data referring to a chronic hCG load are in close agreement with those recently presented by Wang *et al.* (1). These investigators reported a prevalent increase in T levels, with a small increase in E_2, in response to human LH infusion, and no accumulation in C-21 and C-19 precursors. Unfortunately, their study was not carried out while patients were on replacement therapy. Since the testicular response to hCG in HH patients appears to reflect the degree and duration of previous exposure to gonadotropin (16, 17), the testicular steroidogenic responsiveness pattern in HH subjects would be expected to be similar to that in controls after long term gonadotropin replacement. The data presented here show that this was not so, as the pattern remained unchanged even while the patients were receiving hCG replacement. Hence, our data after chronic stimulation with hCG complete those of both Wang *et al.* (1) and Smals *et al.* (2), who, by acute hCG stimulation, demonstrated qualitative deviations in the testicular response in HH subjects.

In man, the injection of a pharmacological dose of hCG induces an initial acute rise in serum T, followed by a plateau for 24–48 h despite high serum hCG; this is evidence of a temporary and partial inability of Leydig cells to respond to hCG with an increase in T production (desensitization). It is widely accepted that such desensitization of Leydig cells accounts for their subsequent inability to respond to additional repeated hCG injections with an increase in T (15, 18, 19). Accordingly, the lack of a further increase in serum T after 8 months of therapy, despite continuous hCG administration, might then be explained by the occurrence of partial testicular desensitization to hCG in HH subjects exposed to chronic treatment with hCG. Indirect evidence of self-regulation of testicular responsiveness to gonadotropin has been suggested by the fact that male patients with gonadotropin-producing tumor have normal plasma T levels in spite of the large amounts of hCG produced by these tumours (20, 21). This seems to suggest that there exists a negative regulation of Leydig cell responsiveness by high concentrations of circulating hCG.

Another plausible explanation for the lack of further T increment during hCG therapy might be that the testes of HH subjects have limited steroidogenic capability due to the presence of an inadequate enzyme system.

The pattern of T response to the injection of 2000 U hCG is not easy to explain. Hypothetically, it could be ascribed to a temporary mechanism of refractoriness of the testicular response, most likely induced by chronic hCG administration. One might postulate that the T increase observed 48 h after the administration of 2000 U hCG may represent resensitization of the testicular responsiveness to the last injection of 1500 U hCG, with a peak occuring 72–96 h after injection. In fact, Glass and Vigersky (15) found that the resensitization phenomenon, with normal rises in 170HP and major T release, takes place 72 h after the injection of a desensitizing dose of hCG.

Hence, the finding of a T response to 2000 U hCG would further favor the idea that desensitization did indeed occur in HH subjects receiving chronic treatment with hCG.

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